Helminth-allergy associations in rural and urban Uganda: insights from antibody studies

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Dedicated to Eva, Silver, Florence, Gloria, Monica, Isabella, Jerome, Yvonne and Martina, who are some of my most favourite people in the world.

DECLARATION

I, **Gyaviira Nkurunungi,** declare that this thesis has been written by me, is an original report of my research and has not been submitted in any previous application for a degree or other professional qualification. Except where it is acknowledged, or stated otherwise by reference, the work herein is my own. Parts of this work have been published as open access articles in *Clinical and Experimental Allergy*, *Parasite Immunology*, *Transactions of the Royal Society of Tropical Medicine and Hygiene, Allergy* and *Clinical Infectious Diseases*. One research paper is In Press (*Scientific Reports*), while another has been submitted to the *Journal of Allergy and Clinical Immunology.* My contribution (and that of co-authors) to these manuscripts is explicitly indicated in this thesis.

Gyaviira Nkurunungi

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ABSTRACT

Background

Allergy-related disease (ARD) is a major cause of morbidity in high-income countries (HICs). Although populations in tropical low-income countries (LICs), and in rural (compared to urban) settings, seem to be remarkably protected, the trends are fast changing as these communities undergo an epidemiological transition exemplified by changing helminth exposure patterns. Helminth exposure is of particular interest in understanding the epidemiology of ARD because of the homology between several helminth antigens and allergens, and the many similarities between helminth- and allergen-specific immune responses. Antibodies, especially IgE, are key constituents of the analogous immune responses to helminths and allergens, and are among the chief mediators of the effector cell activation underlying ARDs. While mindful of effects of other environmental exposures on epidemiological trends of ARDs in LICs, this PhD project postulated a crucial role for helminth-induced antibody profiles.

Methods

This research project obtained data on current helminth infections and allergy-related outcomes, and measured helminth- and allergen-specific antibody profiles, with immunoassays that included allergen and carbohydrate microarrays. Samples used were collected from Ugandan participants of three cross-sectional surveys: the baseline and outcome surveys of a cluster-randomised trial of intensive versus standard anthelminthic treatment in rural *Schistosoma mansoni (Sm)*-endemic islands, and a parallel survey in proximate urban communities with lower helminth exposure. A case-control study on asthma among schoolchildren enabled assessment of a role for antibodies in allergic disease.

Results

Cross-sectional analyses showed that setting (rural vs. urban) was an effect modifier for risk factors (such as location of birth, current helminth [*Sm*] infection and other helminth-related factors) for atopy and clinical allergy outcomes, and for atopy–clinical allergy associations. Although *Sm* infection was an important risk factor for skin prick test (SPT) reactivity and allergen-specific (as) IgE sensitisation (inversely or otherwise), definitive statistical proof for a role of helminth exposure in the observed effect modification between the two settings was not evident: helminths alone may not fully explain the differences observed.

To obtain further insight into helminth-allergy associations, this project assessed total, *Sm*- and asIgE and asIgG4 and found strong positive associations with current *Sm* infection and atopic sensitisation (but not with ARD), but inverse associations between total IgE/ asIgE ratios, asIgG4/ asIgE ratios and SPT reactivity and asthma. This supported a role for helminth-induced antibodies in individual positive helminth-atopy associations, and for the IgG4–IgE balance and the total IgE–allergen-specific IgE balance in the low overall prevalence of clinical allergies in such settings.

Helminths have a range of antigens that are strikingly homologous to common allergens, including cross-reactive carbohydrate determinant (CCD) N-glycans carrying core β-1,2-xylose and α-1,3-fucose epitopes. Analyses showed distinctive relationships between IgE/IgG reactivity to these motifs and *Sm* infection intensity and the rural (versus urban) environment, and implied that they are abundant on common allergen extracts (such as house dust mite, German cockroach and peanut extracts used in standard ImmunoCAP assays), which likely results in false diagnosis of allergic sensitisation in tropical helminth-endemic settings. Microarray component-resolved IgE analyses showed that rural participants had lower responses to non-glycosylated, established major allergenic protein components, but higher reactivity to CCDs, than urban participants. Elevated anti-CCD IgE did not translate into clinical allergy. Indeed, an inverse association between asthma and reactivity to core α-1,3-fucose substituted N-glycans suggested

that reactivity to specific (but not all) CCD epitopes might contribute to protection against clinical allergy.

Conclusions

The current PhD research provides important insight into mechanisms underlying the complex epidemiological helminth-allergy trends in LICs. Notably, this work highlights the importance of balance in IgG4–IgE and total IgE–asIgE in inhibition of clinical allergy. This work also demonstrates strong associations between *Sm* exposure and anti-CCD IgE, which complicates atopy assessment, with implications for understanding the contribution of atopy to ARD in tropical LICs. The finding that schistosomiasis-associated core α-1,3-fucose-specific IgE is inversely associated with asthma is suggestive of a potential role of specific carbohydrate epitopes in protection against clinical allergy, which merits further investigation.

PREFACE

This thesis is written in the 'Research Paper style', in accordance with guidelines provided by the London School of Hygiene and Tropical Medicine. The results chapters consist of four papers that have either been published or submitted for publication in peer-reviewed journals, and which describe work emanating from my PhD studies. In this thesis, each paper is preceded by a cover sheet that provides publication details and states my contribution (as first author) to the work therein. Between the research papers is 'linking material' that comprises supplementary material (published or to be published online with the papers), related unpublished material (if any) and a preamble describing how each chapter (paper) logically leads to the next. Three more papers are presented as appendices to this thesis. All published papers included in this thesis are open access articles of which the authors retain the copyright. The remainder of the thesis comprises background literature to the research conducted, details of the clinical, laboratory and statistical methods, and a general discussion and summary of the research findings. All material was written by Gyaviira Nkurunungi.

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procedures, the Royal Society of Tropical Medicine and Hygiene (RSTMH) for providing me with a small grant award to conduct glycan microarray experiments, and the European Academy of Allergy and Clinical Immunology (EAACI) for the short-term research fellowship that enabled me to perform allergen microarray experiments. Many thanks to the Makerere University – Uganda Virus Research Institute Centre of Excellence for Infection and Immunity Research and Training (MUII-plus), which offered me an honorary fellowship, thereby allowing me to benefit from its invaluable peer support structure.

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LIST OF ACRONYMS

α3Fuc	Alpha-1,3-fucose
β2Xyl	Beta-1,2-xylose
AMC	Amsterdam University Medical Centers
APC	Antigen presenting cell
APCDR	African Partnership for Chronic Disease Research
ARD	Allergy-related disease
asIgE	Allergen-specific IgE
Breg	Regulatory B cell
CCA	Circulating cathodic antigen
CCD	Cross-reactive carbohydrate determinant
DALYs	Disability-adjusted life years
DAMP	Damage-associated molecular pattern
EAACI	European Academy of Allergy and Clinical Immunology
ELISA	Enzyme-linked immunosorbent assay
HCA	Hierarchical cluster analysis
HDM	House dust mite
HIC	High-income country
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
ILC2	Type 2 innate lymphoid cell
IFN-γ	Interferon gamma
ISAAC	International Study on Allergy and Asthma in Children
ISAC	Immuno-solid-phase allergen chip
ISU	ISAC standardised units
IVac	Immunomodulation and Vaccine Programme
KK	Kato-Katz
LaVIISWA	Lake Victoria Island Intervention Study on Worms and Allergy-related Diseases
LIC	Low-income country
LMIC	Low- and middle-income country
LSHTM	London School of Hygiene and Tropical Medicine
LUMC	Leiden University Medical Center

MFI	Mean fluorescence intensity
MRC	Medical Research Council
MS	Mass spectrometry
MUII-Plus	Makerere University – Uganda Virus Research Institute Centre of Excellence for Infection and Immunity Research and Training
PCA	Principal component analysis
PCR	Polymerase chain reaction
RSTMH	Royal Society of Tropical Medicine and Hygiene
SEA	*Schistosoma* egg antigen
Sm	*Schistosoma mansoni*
SPT	Skin prick test
SWA	*Schistosoma* adult worm antigen
TGF-β	Transforming growth factor - beta
Th1	T helper 1
Th2	T helper 2
Treg	Regulatory T cell
UVRI	Uganda Virus Research Institute

CHAPTER 1. BACKGROUND

1.1 The global burden of allergy

Worldwide, allergy-related diseases (ARDs) are important public health problems.[1] Conditions such as asthma, rhinitis and drug and food allergies can be fatal.[2-4] Additionally, these and other conditions such as eczema, conjunctivitis, urticaria, angioedema and insect allergies represent some of the most important causes of chronic morbidity. The prevalence of ARDs increased rapidly in the last century, to the extent that, currently, allergic rhinitis,[1,5] asthma[1] and food allergies[6] affect approximately 400, 300 and 200 million people globally, respectively, and 5-30% of children have eczema.[7-9] Disability-adjusted life years (DALYs) lost due to ARD are substantial. For example, DALYs lost due to asthma were approximately 26 million in 2015, contributing approximately 1.1% of the global burden of disease,[10] and ranking asthma 23rd out of 310 diseases in a 2015 report of causes of disease burden globally.[11] Between 2005 and 2013, dermatitis (atopic, contact, and seborrheic) and urticaria contributed 9.3 million and 4.7 million DALYs lost, respectively, a contribution of 0.4% and 0.2%, respectively, to the global disease burden.[12] The highest prevalence of ARDs has been reported in high-income countries (HICs); however, current time trends point to a sharp increase in low- and middle-income countries (LMICs) as well,[13] including those in tropical sub-Saharan Africa.[1,14] Due to ill-equipped health sectors, low-income countries (LICs) bear the greatest burden of mortality associated with ARD. For example, the mortality rate among asthmatics is higher in LICs than in HICs.[15] Despite these trends, populations in LICs, and in rural (compared to urban) settings, seem to have remained relatively protected against allergies.[16-20]

1.2 The "old friends hypothesis"

Approximately 30 years ago, David Strachan analysed data from a survey of over 17000 British children and observed that younger siblings from larger households seemed protected against hay fever.[21] He postulated that this was possibly because these children had greater exposure to childhood infections, resulting from unhygienic contact

with older siblings. Following these observations, the term 'hygiene hypothesis'[22,23] was coined. According to this hypothesis, the rise in ARDs may have resulted (at least in part) from a decrease in exposure to infections. Assessment of epidemiological trends in HICs provided substantial support for the 'hygiene hypothesis': a review by Jean-François Bach showed that between 1950 and 2000, a dramatic reduction in the incidence of infectious diseases was paralleled by an equal rise in the incidence of autoimmune and allergy-related diseases.[24] Further support came from studies assessing associations between allergy-related conditions and the traditional farming setting (and related microbial exposures) in HICs. For example, in a rural European birth cohort, exposure to animal sheds was dose-dependently inversely associated with wheezing.[25] The United States Amish population, who have maintained traditional farming methods, have a significantly lower prevalence of asthma and allergic sensitisation compared to the Hutterite population, who are of similar genetic descent but have adopted industrialised farming.[26] A remarkable increase in atopic sensitisation was reported among individuals from rural communities in south-west Poland, following an equally significant reduction in farm-related exposures in the same population nine years after Poland's accession to the European Union.[27] Importantly, the prevalence of atopy did not change significantly in small-town populations in the same region. More studies in HICs report comparable results.[28-31]

Common understanding of the hygiene hypothesis, that infectious diseases play a direct role in protection against inflammatory conditions (including allergies),[24] is perhaps inaccurate.[32] In a large study by Benn *et al*.[33] in a Danish birth cohort, infectious diseases were not protective against atopic dermatitis; in fact, positive associations were observed. By contrast, family size and farm residence were protective, in line with the above-mentioned studies.

More recent evidence from epidemiological and experimental human and animal studies backs a different hypothesis, the 'old friends hypothesis'. It is postulated that many

organisms, 'old friends'[34] that co-evolved with mammalian species, developed mechanisms to modulate the expression of inflammatory molecules, as a way of ensuring survival and reproductive success within the dominion of a vigilant host immune system.

The close evolutionary relationship between these 'old friends' and mammals is epitomized by helminth infections: for the most part of history, at least one type or another of these complex multicellular organisms has colonised mammalian species.[35] In HICs, the large reduction in helminths (albeit alongside other infectious agents) during the epidemiological transitions of the last century overlapped with the surge in inflammatory conditions such as allergies,[34] affirming the 'old friends hypothesis'. Tropical LICs still harbour the largest burden of parasitic helminth infections.[36] In these countries, several studies[37-45] have shown that exposure to helminths is associated with protection against allergy and may contribute to urban-rural differences in the prevalence of allergy-related diseases.[46] In Uganda, reports from a birth cohort[47] showed that maternal hookworm infection modifies risk factors for childhood eczema, suggesting that exposure to helminths early in life may also establish protection against allergy.[48]

1.3 Global estimates of disease burden due to helminths

Although better hygiene practices and mass anthelminthic interventions are contributing to a decline in helminths in the most affected regions of the world,[49] World Health Organisation reports estimate that approximately a quarter of the world's population is still infected with at least one helminth.[50] Globally, over 200 million people are infected with schistosomiasis, while approximately 800 million people are estimated to be infected with at least one soil transmitted helminth (STH).[50] In 2010, up to 5.2 million DALYs were lost globally due to STH infections, while schistosomiasis and lymphatic filariasis accounted for approximately 3.3 million and 2.8 million DALYs, respectively.[51,52] The majority of helminth infections affect resource-restrained regions. Over a billion people infected with at least one helminth reside in the Americas, Asia or sub-Saharan Africa.[53]

Helminth-associated morbidity imposes a huge disease burden in infected individuals. For example, chronic *Schistosoma* infections can cause extensive intestinal and liver pathology,[54] and have been significantly associated with anaemia, diarrhoea and undernutrition.[55] Despite the heavy disease burden attributed to helminths, there is evidence of wider implications for protection against non-communicable diseases (such as clinical allergies, discussed herein and in Research paper 5, **Appendix 1**, this thesis), as well as for susceptibility to communicable diseases (Research paper 5).

1.4 Cellular and humoral mechanisms of helminth-allergy associations

Helminths typically induce Th2-type immune responses, characterised by increased levels of interleukin (IL)-4, IL-5, IL-13 and immunoglobulin E (IgE).[56,57] High levels of these responses have also been associated with immunity against several helminth parasites.[58-61] Akin to helminth infections, allergy-related conditions are associated with heightened Th2-type responses.[62] These responses are involved in the initiation of airway smooth muscle contraction,[63] eosinophil recruitment and development,[64] mucus hyperproduction[65] and production of allergen-specific IgE (asIgE) that mediates effector cell degranulation.[66] Similarities between allergen- and helminth-specific immune responses may explain observations, in some instances, of positive helminth-allergy associations[67] Potential mechanisms are covered extensively in sections 1.4.5 and 1.4.6.3, below.

These similarities notwithstanding, helminth infections seem to be protective against allergy-related phenotypes.[38,42-45,68] This has been attributed to the strong and complex network of immunoregulatory mechanisms[69,70] aimed at blocking the allergy-like responses induced by helminths,[71] and which serve to avert helminth destruction and elimination from the host. Evidence of the ability of helminths and their products to modulate immune responses is derived from animal studies and *in vitro* immunological studies in humans, which further demonstrate spillover effects on bystander antigens,[72] including allergens.[73]

The proposed cell- and antibody-mediated mechanisms through which helminths may down-modulate, or enhance, allergic responses are extensive, spanning almost the entire spectrum of the allergy-related immunological pathway. Research paper 5 (**Appendix 1**, this thesis) includes a review of how helminths manipulate host systems, with a schematic (**Figure 1, research paper 5**, reproduced below as **Figure 1.1**) on how bystander responses (including allergy) may be influenced by helminth-host interactions.

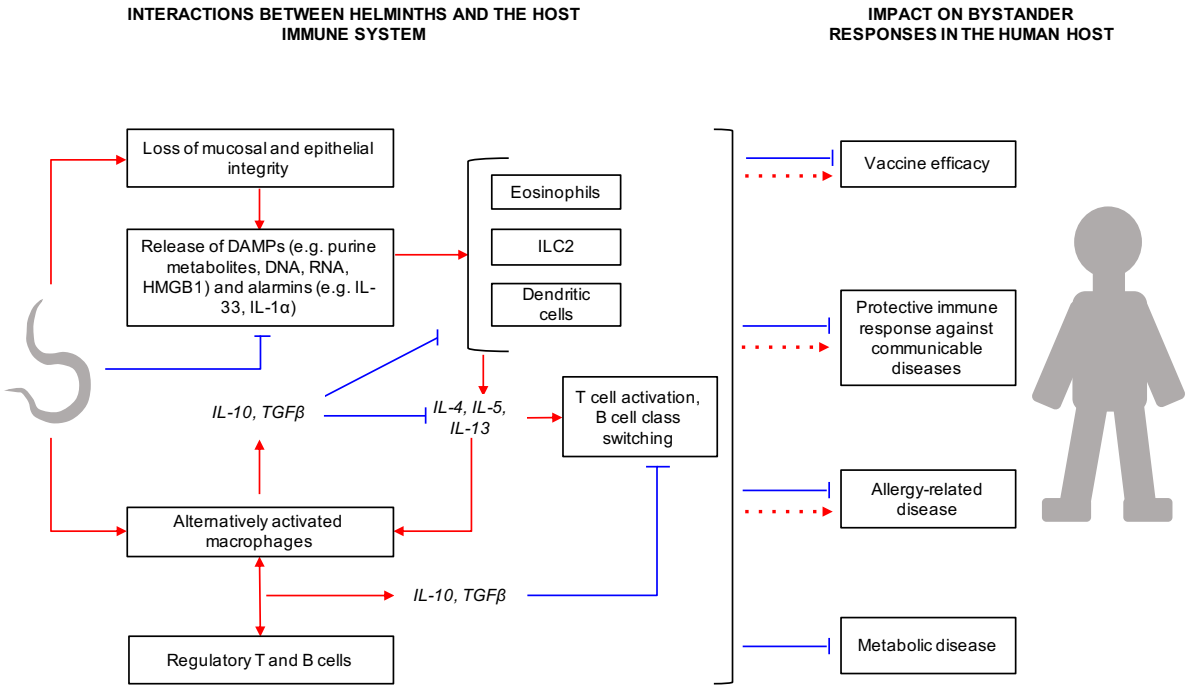

Figure 1.1. Interactions between helminths and the host immune system, and the impact on bystander responses. Red arrows and blue lines denote positive and suppressive effects, respectively. Helminth migration in the host results in tissue injury, resulting in release of Damage Associated Molecular Patterns (DAMPs) and alarmins. DAMPs and alarmins are involved in the initial activation of eosinophils, type 2 innate lymphoid cells (ILC2) and antigen presenting cells (APCs) such as dendritic cells (DCs), which then mediate further inflammation in the host. However, some helminth secretory products can suppress alarmin release and DC maturation, and some helminth enzymes degrade DAMPs. Helminths also interfere with APC activities, promoting an alternative activation phenotype, which results in production of large amounts of IL-10 and TGFβ. These cytokines downmodulate eosinophil, ILC2 and DC responses, and promote lymphocyte hyporesponsiveness involving regulatory lymphocytes. Helminth interaction with host immunity has spillover effects on responses to bystander antigens. For instance, helminth infections may result in impaired immune responses to vaccines and communicable diseases, although specific helminth molecules may actually have enhancing effects. Likewise, there is evidence for both inverse and positive helminth-allergy associations, although any notable effects on metabolic conditions have been beneficial. DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; HMGB1: High Mobility Group Box 1. **Figure reproduced from Research Paper 5, Appendix 1, this thesis**

The present section (1.4) expounds the principal mechanisms of helminth-allergy

associations, with a bias towards antibody-mediated mechanisms.

1.4.1 Interaction of helminths with innate immune cells

Loss of mucosal and epithelial integrity resulting from helminth migration or encounter with allergens and other toxins triggers release of damage-associated molecular patterns (DAMPs) and alarmin cytokines such as IL-1α and IL-33.[23] DAMPs and alarmins are involved in the initial activation of antigen presenting cells (APCs) such as dendritic cells, which then mediate further inflammation in the host. Helminth secretory products have been shown to suppress alarmin release[74] and to secrete enzymes that degrade DAMPs.[75,76] Further downstream, they interfere with APC activities, promoting an alternative activation phenotype, which results in innate cells that produce large amounts of IL-10 and TGF-β but insignificant levels of pro-inflammatory cytokines.[77,78] The modulation of APC function by helminths mainly occurs through the interaction of helminth-derived molecules with pattern recognition receptors (PRRs),[79-82] and has been associated with reduced risk of allergic reactivity in both humans[83] and mice.[84]

Approximately nine years ago, type 2 innate lymphoid cells (ILC2) were recognised.[85] Important as an innate source of Th2 cytokines during helminth infection,[85] ILC2 cells have additionally been implicated in initiation of allergy.[86,87] There is also evidence of potential inhibition of ILC2 activity by *Heligmosomoides polygyrus*,[74] demonstrating the broad nature of helminth-mediated innate immunomodulation, which also includes inhibition of neutrophil activation[88,89] and eosinophil recruitment.[90]

1.4.2 Regulatory T and B cells

Helminths are associated with profound T cell hyporesponsiveness involving regulatory T cells (Tregs), with related increases in interleukin (IL)-10 and transforming growth factor (TGF)-β.[91] Helminth-induced reduced T cell activity may impact on allergy-related outcomes: Navarro *et al.* observed that hookworm anti-inflammatory protein-2 suppressed airway inflammation in a mouse model of asthma, in a Treg-dependent manner,[92] and IL-10 induced during chronic schistosomiasis infection among Gabonese

children was shown to be associated with lower prevalence of atopy.[45] Besides, in both mice[93] and humans,[94,95] IL-10 and Tregs have been implicated in the mitigation of allergy through allergen-specific immunotherapy.

Helminths also induce B cells with various regulatory properties.[96-98] First characterised among helminth infected multiple sclerosis patients in 2008 by Correale and colleagues,[98] these 'regulatory B cells' (Bregs) also produce IL-10, are potent producers of IgG4,[99] and have been associated with inhibition of allergic inflammation.[96,98,100] It is not very clear where in the allergy cascade B and T cell-derived IL-10 applies its modulatory action. IL-10 may impede activities of antigen presenting cells,[101,102] and in an autocrine manner, inhibit T lymphocyte proliferation and activation by suppressing IL-2 production.[103] In addition to influencing lymphocyte and APC activity, reports indicate that IL-10 could inhibit IgE-effector cell degranulation[104] and hence limit the associated allergic inflammation.[45,105]

1.4.3 IgG4-mediated regulation of IgE responses

By inducing high levels of IL-10, helminths may promote immunoglobulin class switching to IgG4. Moreover, chronic helminth infection is associated with elevated serum IgG4 levels.[106] Several studies highlight the importance of helminth-induced IgG4 in allergy: serum inhibition of specific IgE was associated with competing IgG4 in a *Schistosoma mansoni* endemic setting,[107] while in allergen immunotherapy, high IgG4:IgE ratios have been shown to be important.[108] Several possible mechanisms may explain IgG4-mediated protection against IgE-mediated effects. First, IgG4 may block allergen recognition by IgE, because both antibodies have similar antigenic specificity.[107,109,110] Secondly, simultaneous binding of the IgE receptor (FcεRI) and the inhibitory IgG receptor (FcγRIIB) by IgE and IgG4, respectively, may result in a FcγRIIB-dependent inhibition of IgE-mediated effector cell activation.[110-112] Alternatively, IgG4 might not have a direct mechanistic role in protection against allergy-related outcomes. Instead, the inverse association between IgG4 levels and IgE effector function may represent an

'epiphenomenon', merely reflective of the abundance of IL-10 (and/or TGF-β)-producing Tregs and Bregs.[113]

1.4.4 Polyclonal IgE

There is sufficient evidence to link parasitic helminths to stimulation of polyclonal IgE synthesis in humans, including from studies conducted decades ago.[114] For example in 1979, Turner and colleagues showed that *Ascaris*-specific IgE contributed a small proportion of total serum IgE induced by *Ascaris lumbricoides*, suggesting that this parasite mediates production of high levels of IgE that is not specific to the parasite, or to inhalant allergens.[115] Non-specific polyclonally-stimulated IgE has been proposed to inhibit allergic responses by competing with allergen-specific IgE (asIgE) to saturate IgE receptors,[116] reducing the chances that an allergen will result in cross-linking of FcεRI-bound IgE and hence effector cell degranulation.[117,118] Nonetheless, there is contrasting evidence linking high IgE titres to increased expression of IgE receptors on human basophils,[119] implying that high levels of helminth-induced polyclonally-stimulated IgE may potentiate increased effector cell degranulation. Furthermore, Mitre and colleagues showed that ratios of polyclonal to asIgE have to exceed 500:1 to suppress basophil histamine release,[120] yet there is no formal proof of these ratios frequently reaching such high levels during helminth infection. Therefore, the question of whether polyclonally stimulated IgE mitigates allergic responses remains unresolved.

1.4.5 Cross-reactivity between helminth and allergen proteins: implications for allergic sensitisation and allergic effector responses

Although many studies report reduced prevalence of allergy-related conditions in helminth-infected individuals compared to uninfected controls, contradictory evidence (including that shown in **Research Paper 6, Appendix 2,** this thesis) suggests that in some circumstances helminth infections may actually promote allergic responses.[121-124] Helminth-specific immune responses can manifest as allergy-like phenomena, such as Loeffler's syndrome in response to *A. lumbricoides*,[125] cutaneous larva migrans in hookworm infection,[126] 'larva currens' caused by *S. stercoralis*[127] and urticarial rash

induced by several helminths.[128] Urticarial reactions have also been observed during vaccination against hookworm[129] and in response to helminth antigens following treatment.[130]

In addition, protein allergen structures can predict IgE binding to helminth allergen-like proteins,[131] and helminths secrete many proteins that are homologous to known allergens, meaning that cross-reactive helminth protein-specific IgE may induce allergic effector responses. For example, tropomyosin from *Ascaris lumbricoides*[132] and *Onchocerca volvulus*[133] is homologous to tropomyosin from *Dermatophagoides pteronyssinus* (Der p 10)[132] and cockroach (*Blattella germanica,* Bla g 7),[134] and induces basophil histamine release.[133] Immunoglobulin E sensitisation to the *Ascaris lumbricoides* tropomyosin Asc l 3 has been shown to be a risk factor for asthma in Colombian individuals.[135] Other molecules such as paramyosin,[136] glutathione-S-transferase[137] and ABA1-like-protein[132] have also been identified as homologous to allergen proteins known to induce IgE-mediated hypersensitivity. As a result of such cross-reactivity, helminth protein-induced IgE may have implications for serologic-based assays used in diagnosis of allergic sensitisation.[138]

1.4.6 Carbohydrate-specific IgE and allergy

While the role of protein cross-reactivity in helminth-allergy associations has been extensively studied, the importance of cross-reactive carbohydrate groups is less understood. A substantial body of evidence shows that carbohydrates are strong inducers of Th2-type responses.[139-141] Asparagine (N)- and threonine/serine (O)-linked glycans are some of the most common environmental immune determinants,[142-146] comprising a vast number of epitopes for antibodies,[147-150] including IgE.[141,151] Glycans were first linked to allergy in 1981, when Aalberse and colleagues,[152] in a study among venom- and pollen-allergic patients, showed that treatment of food and insect extracts with periodate (which destroys carbohydrate structures) annulled the initially observed cross-reactivity between patient serum IgE and these extracts. Twelve years later, the

α-1,3-fucose moiety on the N-glycan core (trimannosyl-chitobiose, $Man_3GlcNAc_2$)[153] was shown to be majorly responsible for the IgE cross-reactivity between plant and insect glycans.[154] It is now known that some of the commonest sources of cross-reactivity between allergens and helminths are core modifications on N-glycans expressed by insect, plant and helminth proteins (termed cross-reactive carbohydrate determinants, CCDs).

1.4.6.1 Expression of classical CCD epitopes in plants and human parasites

While the N-glycan trimannosyl-chitobiose core ($Man_3GlcNAc_2$) is conserved in all eukaryotes, mass spectrometry (MS)-based analyses of classical CCD N-glycans show two standout, non-mammalian substitutions[145,153] on the $Man_3GlcNAc_2$ core: an α-(1,3) linkage of fucose to the asparagine-linked N-acetylglucosamine (GlcNAc) and a β-1,2-xylose linkage to the first mannose of the trimannosyl component (**Figure 1, Research Paper 3** [this thesis], reproduced below as **Figure 1.2**).

Though absent in mammals, core α-1,3-fucose and β-1,2-xylose motifs are expressed by invertebrate[155,156] and plant glycoproteins,[157-159] as well as on surface and secreted antigens of schistosomes[160] and the nematodes *Haemonchus contortus* and *Caenorhabditis elegans.*[161-164] These core modifications have so far not been detected on glycoproteins from other helminths that are prevalent in tropical regions.[165] Amongst human helminth parasites, the *Schistosoma* glycome is the most characterised.[165-167] Comprehensive mass spectrometry-based studies have detected neither core β-1,2-xylose nor core α-1,3-fucose substituted N-glycans in the adult worm stage of schistosomes; however, both core substitutions are expressed at the miracidia and egg stage. Core β-1,2-xylosylated N-glycans are expressed by cercariae, while α-1,3-fucose substitutions have not been found at this life stage.[165]

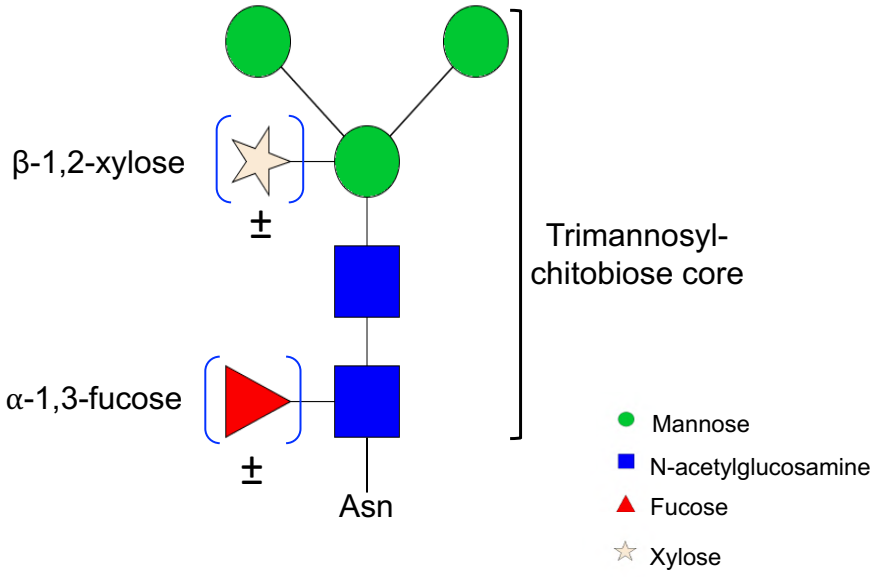

Figure 1.2. Non-mammalian carbohydrate substitutions on the N-glycan core. Non-mammalian monosaccharide substitutions are denoted by blue brackets. **±** implies that motifs in brackets are present or absent in different species. Figure drawn using GlycoWorkbench software, version 2.1 (European Carbohydrates Database Project). **Figure reproduced from Research Paper 3, this thesis.**

There are other common antennary modifications to the schistosome $Man_3GlcNAc_2$ core, which are not established constituents of classical CCD N-glycans. These include antennae composed of GalNAcβ1-4GlcNAc (LDN), GalNAcβ1-4(Fucα1-3)GlcNAc (LDN-F) and Galβ1-4(Fucα1-3)GlcNAc (Lewis X) units. Although these are expressed at all schistosome developmental stages (albeit with varying surface expression patterns),[168] they are uncommon in mammalian species,[169] and are expressed variably by other helminths.[165] **Figure 1.3** (below) summarises the *S. mansoni* life cycle, and expression (or lack thereof) of 1) core β-1,2-xylose and core α-1,3-fucose and 2) common antennary glycan elements, during cercariae, adult worm and egg stages. **Research Paper 3 (Chapter 6)** discusses the role of core substituted glycans and other carbohydrates in diagnostics, cross-reactivity and associations with protective immunity against schistosomes.

1.4.6.2 Clinical relevance of CCDs

Core α-1,3-fucose and β-1,2-xylose may be the most common N-glycan epitopes targeted by human IgE antibodies.[170] Although a few studies have reported that anti-glycan IgE from allergic individuals may trigger histamine release,[171,172] and that the mammalian glycan epitope galactose-α-1,3-galactose (alpha-gal) triggers anaphylactic reactions,[173,174] there is overwhelming evidence showing that carbohydrate-specific IgE generally has insignificant biological activity. For example, individuals with specific IgE to cross-reactive carbohydrate determinants (CCDs) lack SPT and oral reactivity to the same molecules.[118,141,145,175]

Several explanations have been put forward to justify why, predominantly, carbohydrates are benign as allergenic determinants. Early studies proposed that the low affinity of anti-CCD IgE was responsible for its low biological activity;[176,177] however, using surface plasmon resonance technology, it was later shown that human IgE and IgG antibodies against CCDs had high affinity, even comparable to that of protein-specific antibodies.[178] Another factor contributing to the low biological activity of anti-CCD IgE may be the monovalent nature (presence of a single IgE-binding glycan) of most known CCDs (such as bromelain) and other glycosylated allergens. For mast cells or basophils to release histamine or other mediators, two occupied IgE receptors have to be cross-linked by allergens with at least two epitopes. Studies by Mari *et al.*[179,180] showed that bromelain did not induce skin reactivity in individuals with anti-CCD IgE, while poly-glycosylated horseradish peroxidase (HRP) induced skin reactivity in 21% of individuals with CCD-specific IgE. However, in the latter case, reactions were weak, and were mostly observed in individuals with high anti-CCD IgE levels. The extent to which anti-CCD IgE is benign as an allergenic determinant remains poorly understood.

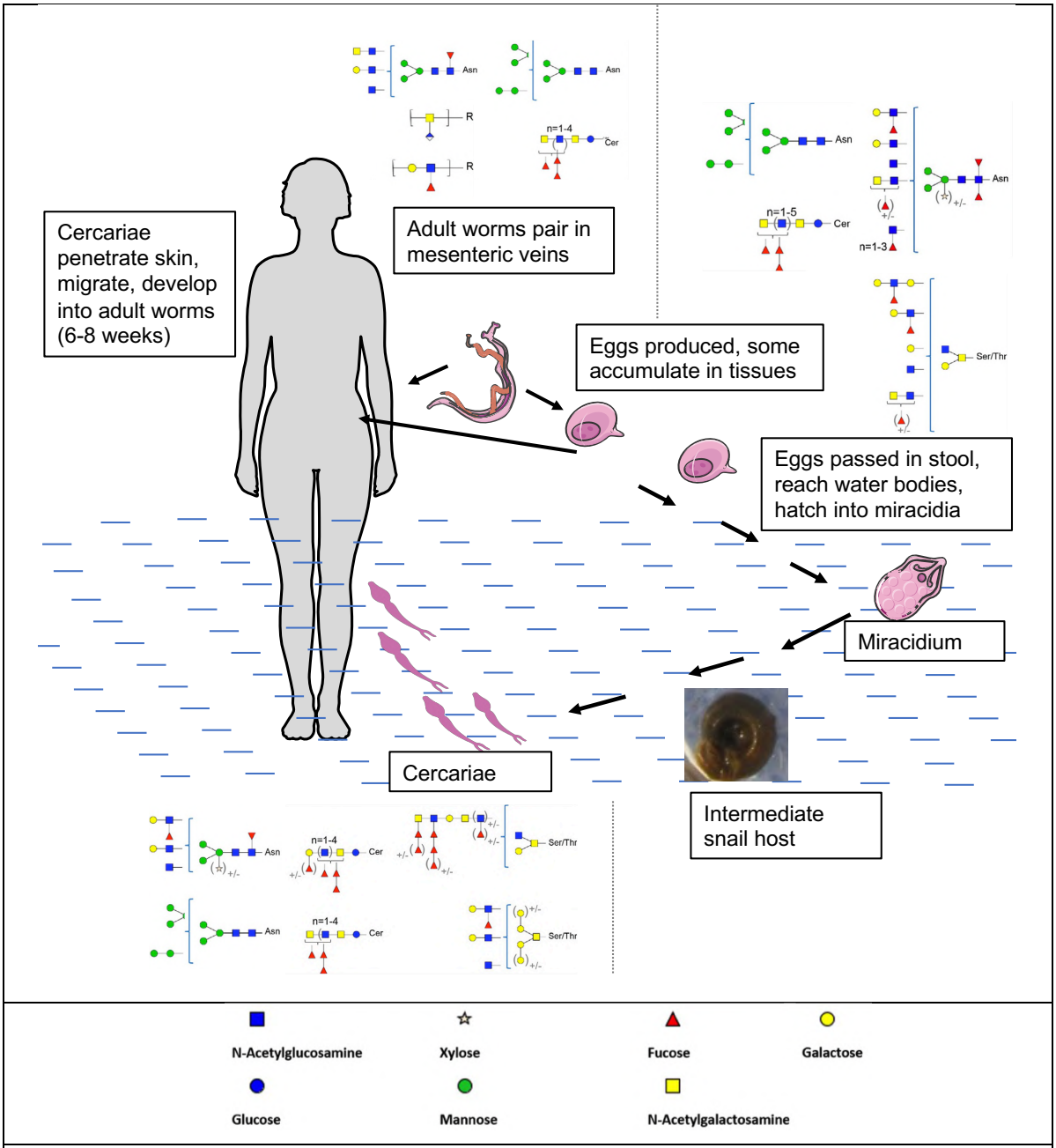

Figure 1.3. *Schistosoma mansoni* life cycle and summary of expression patterns of glycan motifs during the cercariae, adult worm and egg stages. Information on glycan expression patterns obtained from Hokke and van Diepen, 2016.[165] The *Schistosoma* life cycle has been adapted from Elliott *et al*., 2018,[181] with permission from Prof. Elliott, MRC/UVRI and LSHTM Uganda Research Unit. Images of *S. mansoni* life stages and the human body have been adapted from Servier Medical Art (https://smart.servier.com).

1.4.6.3 Anti-CCD IgE and helminth-allergy associations

Cross-reactive carbohydrate determinants on common helminths and allergens may account for elevated (but clinically irrelevant) allergen extract-specific IgE levels in

helminth-endemic areas.[182] Findings by Amoah *et al.*[183] among Ghanaian schoolchildren lend strong support to this hypothesis: *S. haematobium* infection was associated with peanut extract specific-IgE sensitisation, which was in turn strongly associated with anti-CCD IgE but not with SPT reactivity. Additionally, in antibody inhibition assays, IgE reactivity to peanut was inhibited by CCDs and *Schistosoma* egg antigen (SEA). Amoah's results implied that elevated crude peanut extract-specific IgE levels could be attributed to CCDs (expressed by both the crude extract and schistosome antigens), not to true protein allergens in the crude extract. Therefore, in addition to contributing to positive helminth-allergy associations in helminth-endemic areas, elevated anti-CCD IgE levels plausibly obscure the identity of the molecular drivers, and hence interpretation, of atopic sensitisation, especially given that it is routinely diagnosed using allergen extract-based *in vivo* SPTs and/or *in vitro* allergen-specific IgE blood immunoassays.

1.4.6.4 Could carbohydrate-specific antibodies be protective against allergic disease?

Analogous to the idea that "blocking" IgG / IgG4 antibodies compete with IgE to bind to epitopes on allergens,[107,109,110] or that they induce FcγRIIB-dependent inhibition of IgE-mediated effector cell signalling[110-112] (discussed in section 1.4.3), it has been further proposed that high affinity CCD-specific IgG antibodies (resulting from routine contact with foods and pollen) may block IgE-mediated effector responses[184-186] by competing with IgE for allergen epitopes.[187] This represents another potential mechanism of helminth-mediated protection against allergic disease.

Although largely unexplored, a protective role for helminth-induced anti-CCD IgE is also possible. The separation of anti-CCD IgE from clinical symptoms of allergy, coupled with its abundance during chronic helminth infection, raises the question of whether elevated IgE responses to specific immunogenic glycans during chronic helminth infection might dominate over allergen protein-specific IgE, resulting in reduced allergic effector responses. A possible mechanism is that CCD-specific IgE competes with allergen protein-specific IgE to saturate IgE receptors, reducing the chances that allergen

exposure will result in cross-linking of FcεRI-bound IgE to cause effector cell degranulation. It is also plausible that, in an effort by the host immune system to downregulate elevated anti-CCD antibodies, there are spill-over effects resulting in regulation of other responses, including anti-protein IgE. Furthermore, many native allergens occur as glycoproteins; therefore, prior elevated exposure to CCDs (perhaps from helminth infection) may prime initial and recall IgE responses to preferentially target CCD rather than the protein epitopes of allergens. This means that in helminth-endemic settings, IgE is less effectively induced against common protein allergens than against CCDs.

Besides, CCDs may be involved in helminth-mediated immunoregulation: most N-glycans on the *Schistosoma* egg antigen (SEA)-derived glycoprotein omega-1 (which drives both immunoregulatory[188] and Th2 responses,[189] the latter in a glycan-dependent manner[82]) carry core α-1,3-fucose motifs in combination with terminal Lewis X units.[190] Kappa-5, another major SEA component,[191] expresses core β-1,2-xylose and core α-1,3-fucose substituted glycans.[192]

Population studies assessing associations between anti-CCD antibodies and allergic disease, as well as mechanistic studies in animal models and in humans, are required to test the hypothesis that anti-CCD antibodies may be protective against allergic effector responses. It has recently been shown that helminth glycoproteins (including those carrying core substitutions) can be reconstructed in plant systems.[193] Ready availability of tailor-made glycans will be important for mechanistic studies, such as those involving animal models, and could aid development of therapeutics.

1.5 Effect of anthelminthic treatment on allergic sensitisation and disease

Cross-sectional studies are prone to temporality,[194] hence it is important to ascertain causality when assessing helminth-allergy associations. For example, although inverse helminth-allergy associations could result from helminth-mediated protection against allergy-related outcomes, it is also likely that individuals who are genetically predisposed

to allergies could be resistant to helminths. The latter hypothesis draws support from studies which report that specific genotypes are associated with both risk of allergy and resistance to helminth infections.[195,196] Assessing the impact of anthelminthic interventions on allergy-related outcomes is thus an important approach for ascertaining causality when investigating helminth-allergy associations.

Several anthelminthic intervention studies have been conducted to assess effects on allergy-related outcomes. In a study conducted 26 years ago among Venezuelan children, treatment for intestinal helminths was associated with increased SPT reactivity and serum levels of allergen-specific IgE.[197] Notably, among children in the same setting who declined treatment, increased helminth infection prevalence and total IgE levels were observed, paralleled by a decrease in SPT reactivity and allergen-specific IgE levels. In later studies among Gabonese[198] and Vietnamese children[199] repeated anthelminthic treatment was also associated with increased SPT reactivity. However, these studies did not show any effect of anthelminthic treatment on wheeze, eczema or other clinical allergies. Other studies report effects of treatment on some clinical allergy outcomes, but not others, and some studies have observed a lack of effect of treatment on both allergic sensitisation and clinical allergy. For example, Cooper and others found that ivermectin treatment for *Trichuris trichiura* infection among Ecuadorian children was associated with increased prevalence of eczema symptoms (but not asthma symptoms),[200] but did not observe any effect of two-monthly albendazole (for one year) on atopy or clinical allergy in a large, cluster-randomised trial among schools in rural Ecuador.[201]

Barring investigations on the effect of anthelminthic treatment on allergen extract-specific IgE (as a measure of atopy), very few studies have included detailed work on the effect of anthelminthic treatment on immunological parameters related to allergy, such as IgE-mediated histamine release, anti-CCD antibody responses, allergen extract-specific IgG, or allergen component-resolved antibody reactivity. However, one such study was

conducted in Ugandan school children: while hookworm infection abrogated the predicted association between allergen-specific IgE and basophil histamine release, albendazole treatment re-established it.[202] More animal and population-based studies are warranted.

1.6 Helminths, the rural-urban environment and allergy-related outcomes

The on-going urbanisation in many tropical LICs is gradually being paralleled by a shift in disease patterns.[203] Non-communicable diseases, including allergy, seem to be on the increase, especially in urban areas of LICs. Links between urbanisation and allergic disease are not new. As far back as 1965, urban-rural differences in asthma prevalence were being reported among German populations.[204] Later, studies in Africa,[205] Asia[206,207] and South America[208] reported similar results. Factors that are inversely associated with allergy-related conditions have been shown to be more common in the rural environment, as indicated by studies on the protective effect of traditional farming on allergy outcomes (discussed above).[25,26,28-31] Rural settings, especially in tropical LICs, are characterised by exposure to immunomodulating disease agents such as helminths, which have been hypothesised to protect against allergy-related disease.[34] Changes in environmental exposures due to urbanisation may drive increases in allergy-related disease.[34] These changes include reduced exposure to helminths. Previous work implies that there are other potentially relevant differences in the urban-rural exposure that may influence allergy trends, such as animals and related microbial exposure[209] and nutrition (for example milk pasteurisation[27]). However, helminths are of special interest because of their unique molecular relationship with allergens (discussed above). Whether repeated, intensive anthelminthic treatment in endemic rural settings in the tropics may shift allergy prevalence or immunological profiles associated with allergy towards an urban pattern has not been explored fully. While conceivable, such a shift might be hindered by high re-infection rates in most endemic settings[210] and the long-lasting immunological and epigenetic effects of helminth exposure.[211]

1.7 Scope of this thesis

The prevalence of helminth infections is still high in most tropical LICs, especially in rural settings. This, coupled with the ongoing epidemiological transition in these countries, provides unprecedented opportunities to understand helminth-allergy associations, by exploring the underlying immunological mechanisms. In Uganda, the urban-rural divide is characterised by differences in helminth exposure intensity between the two settings, offering opportunities for investigation of the role of helminths (through specific immunological pathways) in mediating population differences in allergy-related conditions.

Antibodies, specifically IgE, are key constituents of the analogous immune responses to helminths and allergens, and are among the chief mediators of the effector cell activation underlying allergy-related disease. Three studies (described in Chapter 3) have been conducted in proximate, yet diverse Ugandan settings, primarily to obtain a comparative assessment of phenotypes, prevalence and risk factors for allergy-related diseases in rural and urban settings, and among asthmatic schoolchildren (age 5-17 years) and non-asthmatic controls. These studies provided a unique resource for assessment of the impact of the rural-urban environment and helminth exposure on helminth protein and carbohydrate-specific antibody profiles and the relevance of these profiles to the epidemiological trends of allergy in tropical LICs, specifically Uganda. These assessments are important for therapeutic strategies against allergy-related disease and for strategies aimed at minimising the impact of improved hygiene on allergy prevalence in tropical LICs.

1.8 Chapter 1 References

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CHAPTER 2. THESIS OBJECTIVES AND STRUCTURE

2.1 General hypothesis

Helminth-induced antibody profiles influence the profile of allergic sensitisation and disease, and consequently the epidemiological trends pertaining to allergy in tropical settings.

2.2 Conceptual framework

The conceptual framework of this thesis is shown in **Figure 2.1**, below.

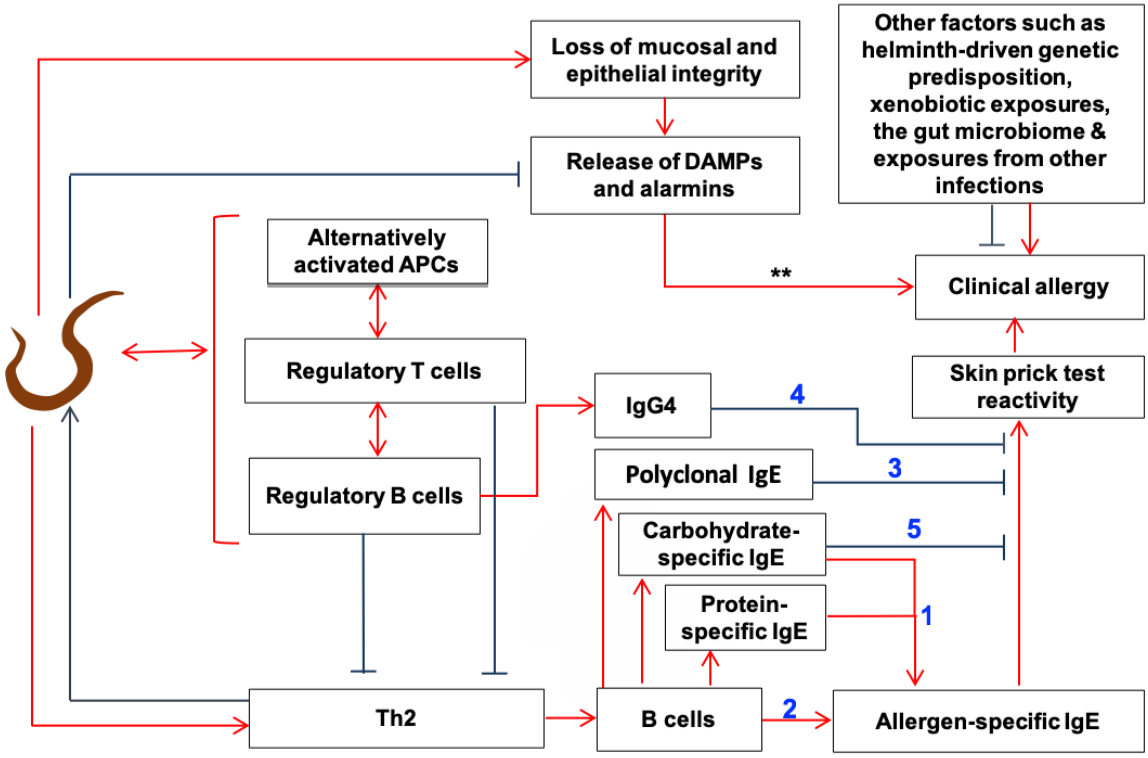

Figure 2.1. Conceptual framework. Red arrows indicate positive effects and blue arrows indicate negative effects. Standard arrow endings denote promotion, while perpendicular line endings denote inhibition. Helminth antigens may cross-react with allergen protein or carbohydrate determinants (1), and/or interact with B cells (2) to elicit strong allergen-specific IgE (asIgE) responses. Conversely, they might non-specifically induce production of polyclonal IgE (which might inhibit allergic responses by competing with asIgE to saturate IgE receptors (3)) or induce IgG4 that will block allergen recognition by IgE (4). Helminths such as *Schistosoma mansoni* contain IgE-binding carbohydrate moieties (also found on some allergen glycoproteins; do not translate into allergic effector responses): during chronic helminth infection, carbohydrate-specific IgE might dominate over protein-specific IgE, resulting in reduced allergic effector responses (5). ******Alarmins such as IL-33 promote Th2-mediated inflammation, including IgE production and eosinophil activation, which may contribute to pathogenesis of some clinical allergies such as asthma.[1] DAMP-induced activation of the inflammasome has been implicated in airway hyperreactivity in asthma.[2]
APCs: antigen presenting cells; **DAMPs**: damage-associated molecular patterns.

2.3 Thesis objectives

1) To investigate the extent to which helminth exposure influences rural-urban differences in prevalence of allergy-related outcomes, and in epidemiological and immunological risk factors for these outcomes

2) To map the association of total, helminth- and allergen extract-specific IgE and IgG4 profiles with helminth infection and allergy-related outcomes

3) To assess associations between helminth (*S. mansoni*) infection (and intensity) and IgE and IgG responses to classical cross-reactive carbohydrate determinant (CCD) epitopes

4) To determine the role of CCD-specific IgE in the epidemiology of allergic sensitisation and disease (asthma)

2.4 Thesis structure

This thesis is written in the 'Research paper' format, and comprises eight chapters. Each of the seven research papers included in this thesis was written as a stand-alone manuscript, albeit using data collected from the same study settings. Therefore, there is inevitable duplication of some of the information among the papers, and between the papers and the remainder of the thesis. Duplicated material includes information on study settings, experimental methods and clinical procedures. The research papers are not presented in the chronological order of publication, but rather in the order in which research objectives are listed above.

Chapter 1 is the thesis background. It presents literature on the burden of allergy-related outcomes in the tropics, the helminth-allergen interaction and similarities in the immune responses associated helminth infections and allergy, current understanding of hypothesised mechanisms of inverse and positive helminth-allergy associations and the potential role of helminth and allergen protein- and carbohydrate-specific antibodies in epidemiological trends of allergy. This section also touches upon current understanding of the interaction between the rural-urban environment and helminth infection exposure

and how this may influence the epidemiology of allergy in the tropics. Finally, the scope of this PhD thesis is given.

Chapter 2 describes the general hypothesis, thesis objectives and the thesis structure.

Chapter 3 provides a detailed account of the study design, study setting, relevant clinical procedures and experimental methods.

Chapter 4 (Research Paper 1)[3] is the first results chapter, and presents findings from cross-sectional analyses of epidemiological and immunological risk factors for atopy and clinical allergy outcomes in urban and rural Uganda. This chapter further assesses to what extent helminth infections contribute to rural-urban differences in these risk factors, in Uganda.

Chapter 5 (Research Paper 2) describes total, allergen- and *S. mansoni*-specific IgE and IgG4 profiles, and their relationship with *S. mansoni* infection status, atopic sensitisation and clinical allergy in a rural setting with high prevalence of *S. mansoni* infection.[4] Although the focus of this thesis is antibodies, this paper also describes associations between *S. mansoni*-specific cytokine profiles and allergy-related outcomes, giving a more complete picture of the association between *S. mansoni*-specific immune responses and allergy in Uganda.

Chapter 6 (Research Paper 3) describes population-level antibody responses to key components of the schistosome glycome: core β-1,2-xylose and α-1,3-fucose substituted N-glycans.[5] This chapter highlights rural-urban differences in these responses, describes their relationship with *S. mansoni* infection and intensity and discusses their relevance to diagnostics, cross-reactivity and associations with protective immunity against *S. mansoni.*

Chapter 7 (Research Paper 4) assesses associations between IgE reactivity to cross-reactive carbohydrate determinant (CCD) N-glycan epitopes (core β-1,2-xylose and/or α-1,3-fucose, introduced in Chapter 6) and allergic sensitisation and disease.

Chapter 8 discusses the main findings from this PhD project, and provides an account of study strengths, limitations and implications. The conclusion and recommendations for further research are also included in this chapter.

Appendices are included after Chapter 8. **Appendix 1** is a published narrative literature review (Research Paper 5)[6] of the benefits and potential detrimental effects of mass anthelminthic treatment with regard to responses to vaccines and unrelated diseases (such as allergies). **Appendix 2** is an original article (Research paper 6)[7] describing epidemiological associations between helminth infections and allergy-related outcomes in the Lake Victoria fishing communities, Uganda, prior to community-wide intensive versus standard anthelminthic treatment. **Appendix 3** is also an original article (Research Paper 7)[8] describing an assessment of the impact of community-wide intensive versus standard anthelminthic treatment on allergy-related outcomes, helminth infection intensity, and helminth-related morbidity in Lake Victoria fishing communities, Uganda. **Appendix 4** includes copies of ethical approvals.

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CHAPTER 3. MATERIALS AND METHODS

3.1 Preamble

This chapter provides an overview of context and methods for the work conducted during this PhD research. Methods are further summarised in the individual Research Papers.

3.2 Study design and population

Most of the data presented in this PhD thesis were obtained using samples from participants of three cross-sectional surveys in two proximate settings in Uganda, one characterised as rural and the other as urban. The first two surveys were 1) the baseline and 2) the allergy outcomes survey of a cluster-randomised trial of community-wide intensive versus standard anthelminthic treatment in helminth-endemic Ugandan fishing villages. The third survey was a parallel survey of allergy outcomes in proximate urban communities with lower helminth exposure. **Chapter 5** (Table E5) and **Chapter 7** further include data from samples obtained from a case-control study among asthmatic children and non-asthmatic controls from urban and peri-urban schools in Uganda. These studies were part of a portfolio of work on the prevalence, phenotypes and risk factors for allergy-related outcomes in Uganda, and are described below.

3.2.1 The LaVIISWA trial

Residents of 26 fishing villages in the islands of Koome sub-county (Lake Victoria, Mukono district, Uganda, **Figure 3.1**, below) were invited to participate in the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA; ISRCTN47196031, described below and in Appendices 2 and 3 in more detail).[1] Koome sub-county had a population of 18,778 in 2014, according to the Uganda National Population and Housing Census report[2]. The living conditions in Koome islands are characterised by poor housing and sanitation (**Figure 3.2**, below), and lack of access to treated water. Helminth prevalence is very high: up to 85% of individuals living in the fishing villages in 2016 were infected with *Schistosoma mansoni* (based on the urine test for circulating cathodic antigen, CCA) and 9% with hookworm (based on PCR detection

of helminth DNA from eggs in stool).[3]

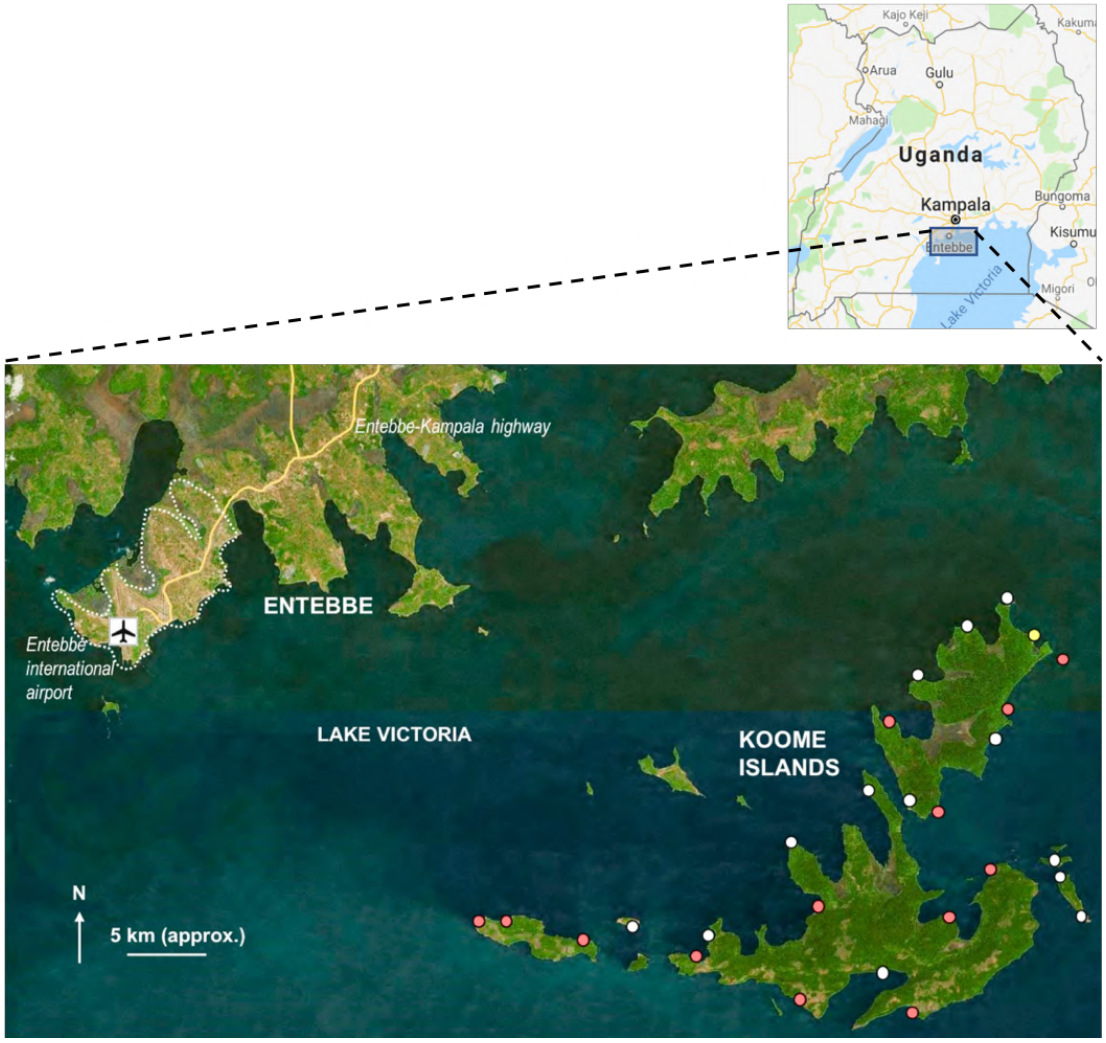


Figure 3.1. Entebbe peninsula and Koome islands. White dotted demarcation on the Entebbe peninsula denotes the boundary of the municipality. Circular features on Koome islands denote fishing villages. White circles represent villages that received community-based standard anthelminthic treatment, red circles represent villages that received intensive treatment and the yellow circle represents the village where the LaVIISWA pilot study was conducted.

The LaVIISWA trial[1] was conducted between September 2012 – August 2016. It was an open, cluster-randomised trial of mass intensive versus standard anthelminthic treatment. Twenty-six villages were randomised 1:1 to receive either intensive or standard intervention. Intensive intervention was quarterly single-dose praziquantel (40mg/kg, estimated using an extended height pole to include younger children) and quarterly triple-dose albendazole (400mg daily, three days) to all individuals that were

one year or older. Standard intervention was annual single-dose praziquantel (40mg/kg, estimated using a standard height pole), and six-monthly single-dose albendazole 400mg to all individuals that were one year or older. There was no follow-up of individual participants, as the study did not include a cohort of individuals. This trial was the first of its kind, and hence provided the first opportunity for population-level evaluation of changes (in antibody profiles) induced by such intervention. A detailed description of the LaVIISWA trial, including study design and setting, randomisation procedures, interventions, surveys and outcomes has been published, and is included in this PhD thesis as Research Paper 7 (**Appendix 3)**.

LaVIISWA data used in this PhD Research were obtained during two cross-sectional surveys. A baseline household survey (October 2012 – July 2013) was conducted before the first round of the trial intervention: epidemiological associations between helminth infections and allergy-related outcomes are reported in **Research paper 6** (Appendix 2, this thesis). **Chapter 5** (Research Paper 2) presents results (on total, allergen- and *S. mansoni*-specific IgE and IgG4 profiles) obtained from samples collected during the baseline survey.

After three years of the trial intervention, an allergy outcomes survey (September 2015 – August 2016) was conducted. Before this survey, a census of households in each village was done. Seventy households per village were then randomly selected for the survey, using a Stata program (College Station, Texas). Household members aged ≥ one year were invited to participate. Permission for household participation was granted by the household head, or another adult, if the former was absent. Results from the outcome survey indicated that intensive versus standard anthelminthic treatment reduced hookworm prevalence and *S. mansoni* infection intensity, but had no effect on the overall prevalence of other nematodes and *S. mansoni,* or allergy-related outcomes (**Research Paper 7, Appendix 3**). Furthermore, there was no evidence of an effect of treatment arm on most laboratory outcomes (**Table E1, Chapter 5**). Therefore,

Chapters 4, 6 and 7 (this thesis) which present results from the 'rural survey' have used data combined from both trial arms of the LaVIISWA outcome survey.

3.2.2 The urban survey

Urban participants were residents of Entebbe Municipality, a lower helminth exposure area situated on the northern shores of Lake Victoria (**Figure 3.1**, above), 40 km southwest of the Ugandan capital, Kampala, and 35km (two to three hours by powered canoe) from Koome islands. The municipality has different living conditions from Koome islands. It is characterised by better housing and sanitation (**Figure 3.2**, below), and access to treated water. The municipality had approximately 69,430 inhabitants in 2014,[2] distributed across two municipality divisions, A and B. Each division comprises two wards that are further subdivided into sub-wards. The municipality has a total of 24 sub-wards, which are its smallest administrative units.

The urban survey of allergy-related outcomes (September 2016 – September 2017) was designed to compare findings with those from the aforementioned high-helminth-transmission rural surveys. The urban survey was designed in such a way that procedures used (including laboratory methods) were identical to those used in the rural LaVIISWA outcome survey; the main difference in study design was the lack of randomisation to intensive versus standard mass anthelminthic treatment in the urban survey. The sampling strategy (described below) was also different, as it was not feasible to map and enumerate all households in Entebbe.

A

B

Figure 3.2. Pictorial representation of living conditions in Koome sub-county and Entebbe Municipality.

A) Photographs of Koome fishing villages. From left to right: Mwooma and Kitosi villages and an aerial view of Myende fishing village.

B) Photographs taken in Entebbe municipality. From left to right: Street view of Entebbe town (Post Office sub-ward), residences in Bugonga sub-ward and an aerial view of Katabi ward

Sources: Photographs of Mwooma and Kitosi villages were kindly provided by Prof. Alison Elliott, MRC/UVRI and LSHTM Uganda Research Unit. The aerial view of Myende village was taken by Prof. Russell Stothard, from the Liverpool School of Tropical Medicine, and is reused with permission. The satellite image of Katabi ward was obtained from Google maps.

Before the start of the survey, each sub-ward was mapped onto satellite images of Entebbe municipality with the help of locally available maps. A random point generation function of ArcGIS software (version 10.4.1, Environmental Systems Research Institute, Redlands, CA) was then used to generate random starting points within each sub-ward. The number of starting points selected was proportional to the population size of the sub-ward. Coordinates of the random starting points generated were loaded onto geographic information system (GIS) devices (eTrex®, Garmin™ Ltd, Olathe, KS). These devices were then used in the field to identify the selected random points, from which the nearest four houses were surveyed.

3.2.3 The asthma case-control study

The asthma study (May 2015 – July 2017) enrolled 5-17-year-old asthmatics and non-asthmatic controls from primary and secondary schools in Entebbe municipality and the surrounding peri-urban and urban areas in Wakiso District in central Uganda. Asthma was defined as a history of wheeze in the last 12 months, according to the International Study on Allergy and Asthma in Children (ISAAC) paper and video questionnaires. All children without a history of wheezing (ever) were eligible as controls for the study, provided that they were on the same class register as cases. For each class, a Stata programme and the class register were used to randomly select participants such that the number of controls was twice the number of cases. Procedures in the asthma study were also designed to be identical to those used in the rural and urban surveys.

3.3 Parasitological examinations

3.3.1 Kato-Katz

The Kato-Katz technique[4] was used for qualitative and semi-quantitative diagnosis of *Schistosoma mansoni* infection and intestinal helminth infections caused by hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*. The technique was conducted on one stool sample per participant, prepared as a 41.7 mg thick smear on two slides. Each slide was

examined under a microscope by different laboratory technologists who were blinded to each other's readings. Results were presented as eggs per gram of stool (epg). *Schistosoma mansoni* infection (Kato-Katz) intensities were categorised according to World Health Organisation (WHO) guidelines as follows: light infection 1–99 epg, moderate infection 100–399 epg and heavy infection ≥400 epg.

3.3.2 Detection of helminth DNA in stool

3.3.2.1 DNA extraction

The remaining stool sample was stored (suspended in ethanol and frozen at -80°C) and later had total DNA (and hence helminth DNA, if present) extracted using the QIAamp DNA Mini Kit (catalogue number 51306, QIAGEN) for determination (using multiplex real-time PCR) of *S. mansoni, Strongyloides stercoralis* and hookworm (*Necator americanus*) infections.

The DNA extraction procedure was conducted with minor changes to the manufacturer's instructions as follows: samples were left to thaw at room temperature (RT) and then vortexed for five seconds to homogenise the ethanol-stool mixture. The homogenized mixture (0.5 ml) was transferred into a safe-lock microcentrifuge (Eppendorf®) tube and centrifuged at 13000 rpm for 3 minutes to get rid of the ethanol. The pellet was re-suspended in 200 µl of 1X phosphate buffered saline (PBS) containing 2% polyvinylpolypyrrolidone (PVPP) (77627, Fluka analytical, Sigma-Aldrich) and frozen overnight at -20°C. The stool suspension was heated at 100°C for 10 minutes, 200 µl of the QIAGEN tissue lysis buffer (ATL) and proteinase K mixture (9 to 1 ratio) added, and samples vortexed and incubated for two hours or overnight at 55°C in a heat block. The QIAGEN AL Buffer (400 µl) was then added, mixed thoroughly with the sample by vortexing and the mixture incubated at 70°C for 10 minutes. This was followed by centrifugation for one minute at 13000 rpm and transfer of the supernatant to 400 µl of ethanol (96-100%). The DNA was then purified in QIAamp spin columns using consecutive washes with QIAGEN AW1 (500 µl) and AW2 (500 µl) buffers. The QIAGEN

AE buffer (200 µl) was used to elute the DNA. The DNA was quantified on a NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific) and then diluted to 50ng/µl.

3.3.2.2 Multiplex real-time PCR

The multiplex real-time PCR was adapted from existing procedures,[5,6] and is detailed in this section. Below are the specific forward (F) and reverse (R) primers and TaqMan® probes that were used to simultaneously detect DNA from three helminth species:

Necator americanus

Na58F: 5'-CTGTTTGTCGAACGGTACTTGC-3'

Na158R: 5'-ATAACAGCGTGCACATGTTGC-3'

Na81MGB: FAM-5'-CTGTACTACGCATTGTATAC-3'-BHQ1

Schistosoma mansoni

Ssp48F: 5'-GGTCTAGATGACTTGATYGAGATGCT-3'

Ssp124R: 5'-TCCCGAGCGYGTATAATGTCATTA-3'

Ssp78T-RT: Texas Red-5'-TGGGTTGTGCTCGAGTCGTGGC-3'-BHQ2

Strongyloides stercoralis

Stro18S-1530F: 5'-GAATTCCAAGTAAACGTAAGTCATTAGC-3'

Stro18S-1630R: 5'-TGCCTCTGGATATTGCTCAGTTC-3'

Stro18S-1586T: NED-5'-ACACACCGGCCGTCGCTGC-3'-BHQ1

Phocine herpes virus (PhHV) DNA, extracted from the Phocine herpes virus (kindly provided by Dr. Martin Schutten, Erasmus Medical Center, Rotterdam, the Netherlands), was included in the PCR master mix, thus distributed to all reaction wells as an internal control to check for PCR inhibition. The PhHV forward primer PhHV-267s (5'-GGGCGAATCACAGATTGAATC-3'), reverse primer PhHV-337as (5'-GCGGTTCCAAACGTACCAA-3') and probe PhHV-305tq (Cy5-5'-

TTTTTATGTGTCCGCCACCATCTGGATC-3'-BHQ2) were used for Phocin herpes virus DNA detection. A positive pool was included on the plate for every run as a test control. The positive pool was made up of a mixture of DNA from samples (from among the study samples) that were highly positive for *S. mansoni* and *N. americanus* on Kato-Katz, and DNA positive for *S. stercoralis* (kindly provided by Dr. Jaco J. Verweij, St. Elisabeth Hospital, Tilburg, the Netherlands). The amplification conditions were 10 minutes at 95°C, 50 cycles of 15 seconds at 95°C, 30s at 60°C and 30s at 72°C. DNA amplification, detection and data analysis were attained with the ABI 7500 Fast Real time machine and 7500 Fast systems software version 1.4.0. Quality control was conducted by Dr. Jaco J. Verweij, using a small subset of DNA samples.

3.3.3 Assessment of circulating cathodic antigen of S. mansoni in urine

The circulating cathodic antigen (CCA) of *S. mansoni* was assessed in mid-stream urine using a point-of-care test from Rapid Medical Diagnostics, South Africa (http://www.rapid-diagnostics.com). The CCA assays were performed according to the assay kit manufacturer's instructions. A result was considered invalid if the control band did not form. Valid results were presented as negative (no test band) or positive (irrespective of whether the test band was weak or strong).

Schistosoma haematobium is not present in the areas where the studies were conducted.[7]

3.4 Assessment of allergy-related outcomes

The main allergy-related outcomes were recent (previous 12 months) self-reported wheeze (as a proxy for asthma), skin prick test (SPT) reactivity to common allergens in the study settings,[8] and allergen-specific IgE (asIgE) sensitisation. Data were also obtained on visible flexural dermatitis, recent rhinitis and recent urticarial rash.

3.4.1 Wheeze

Standardised paper and video questionnaires about wheeze have been shown to have good validity for the diagnosis of asthma in epidemiological studies,[9,10] and hence were used to obtain data on recent (previous 12 months) self-reported wheeze. These questionnaires were interviewer-administered, and used guidelines from ISAAC.[11] Wheeze was analysed separately for individuals below and above age five years. The principal age group of interest was ≥5 years because wheeze among children aged below five years does not necessarily represent asthma:[12] it can be caused by conditions such as viral bronchiolitis.[13]

3.4.2 Visible flexural dermatitis

Visible flexural dermatitis, the classical physical sign of eczema, was assessed by interview using questions from the UK diagnostic criteria on atopic eczema, and by direct physical examination as described in Williams' on-line manual.[14] In brief, an individual was recorded as having dermatitis in case of an erythematous rash with surface change (vesicles, scaling, crusting, oozing, or lichenification) in and around skin creases (fronts of neck or ankles, folds of elbow, behind the knees), or among infants, on the cheeks, the trunk and the outer surface of the limbs.[15,16]

3.4.3 Rhinitis and urticarial rash

Data on rhinitis (runny/blocked nose or sneezing accompanied by watery and itchy eyes, in absence of a cold or 'flu') and urticarial rash (pruritic rash with elevated erythematous patches [wheals], known as 'ebilogologo' in the local Luganda language) were obtained using interviewer-administered questionnaires.

3.4.4 Skin prick test reactivity

Skin prick test reactivity to common environmental allergens in the study settings[8] was assessed using standard procedures.[17] The allergens assessed were *Dermatophagoides* mix, *Blomia tropicalis* and German cockroach (*Blattella germanica*)

(ALK-Abelló; supplied by Laboratory Specialities [Pty] Ltd., South Africa). Individuals were considered reactive if they had a wheal ≥3mm in diameter, in presence of histamine (positive) and normal saline (negative) controls. Reactivity was analysed primarily as a positive SPT response to any of the three allergens versus no response to all three allergens. SPT reactivity was also assessed as a positive versus negative response to individual allergens.

3.4.5 Measurement of total and allergen-specific IgE using the ImmunoCAP test

The ImmunoCAP® test (Thermo Fisher Scientific, Uppsala, Sweden)[18] was used to measure total and crude house dust mite (*Dermatophagoides pteronyssinus*), peanut (*Arachis hypogaea)*, and German cockroach (*Blatella germanica*) extract-specific plasma IgE (asIgE) levels. The ImmunoCAP assay uses an automated system that loads serum / plasma on **1)** a hydrophilic polymer sponge (the 'ImmunoCAP') covalently coupled to an allergen (for specific IgE measurement) or **2)** an ImmunoCAP reaction vessel covalently coupled to an anti-IgE antibody (which reacts with total IgE in sample). Unbound IgE is washed off and a conjugate (anti-IgE antibody grafted with β-galactosidase) is added. The supernatant is then aspirated, and a substrate (methyl-umbelliferyl-β-D galactoside) deposited on the ImmunoCAP sponge. A stop solution is added and the sponge then compressed. The fluorescence generated by the resultant eluate, a product of enzyme degradation (methyl umbelliferone, proportional to concentration of IgE in serum/plasma), is measured. Results are reported quantitatively using a kU/L scale. The calibrator is IgE bound to anti-IgE caps using a six-point quantitative curve. Calibration concentration ranges from 0 to 100 kU/L for specific IgE and 2 to 5000 kU/L for total IgE.

A cut-off of 0.35 kU/L was used to define allergen-specific IgE sensitisation to any of house dust mite, peanut or cockroach, as recommended by the test manufacturer.[19] Responses were also assessed as continuous outcomes and as positive (≥0.35 kU/L)

versus negative for individual allergens. Total IgE was assessed as a continuous outcome.

3.4.6 Measurement of allergen-specific IgE levels by ELISA

Crude *D. pteronyssinus*- and *B. germanica* extract-specific IgE was measured using an in-house ELISA, as follows: all but the first 2 columns of MICROLON® high binding 96-well plates (Greiner bio-one, UK) were coated with 50µl of *D. pteronyssinus* or *B. germanica* allergens (Greer Labs, Lenoir, NC, USA) at a concentration of 5µg/ml in in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer (0.1M, pH 9.6). Two-fold dilutions of human IgE (Calbiochem, Beeston, UK) standard, diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4ºC. Plates were washed with phosphate-buffered saline (PBS)-Tween 20 solution, blocked with 150µl of skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4ºC with 50µl of plasma samples diluted 1/20 with 10% fetal bovine serum in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates overnight at 4ºC with 0.5µg/ml of biotinylated monoclonal mouse anti-human IgE (BD Pharmingen™), followed by a one-hour incubation with a streptavidin-Horseradish Peroxidase (strep-HRP) conjugate (Mast Group Ltd, Bootle, UK), diluted 1/3000 with assay buffer, at RT. Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped with 30µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgE concentrations (ng/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

This assay had a lower detection limit of 15.6 ng/ml (hence 312 ng/ml in undiluted samples). This cut-off was used to assess detectable (versus undetectable) responses to either *D. pteronyssinus* or *B. germanica.* Responses were also assessed as continuous outcomes.

3.4.7 Component-resolved assessment of allergen-specific IgE sensitisation using the ISAC microarray

The ImmunoCAP® ISAC (Immuno Solid-phase Allergen Chip) microarray was used to measure IgE to 112 natural and recombinant allergen components from 51 allergen sources. The ISAC array (Thermo Fisher Scientific, Uppsala, Sweden) is an *in vitro* semi-quantitative multi-allergen screening assay used in component-resolved diagnosis of allergic sensitisation.[20] The test manufacturer's instructions were followed. Briefly, ImmunoCAP ISAC sIgE 112 microarray chips were washed, air dried and then incubated with 30µl of undiluted participant plasma for two hours in a humidified chamber to enable reactions between IgE and allergen components. The chips were washed again, and incubated at room temperature for 30 minutes with 30µl of fluorescence-labelled anti-human IgE detection antibody. After another washing step, the fluorescence intensity of each microarray was measured by a scanner (LuxScan 10K/A, CapitalBio, Beijing, China). Analysis of the digitalized images was done using Phadia Microarray Image Analysis software (Thermo Fisher Scientific). Results were reported in arbitrary semi-quantitative ISAC Standardised Units (ISU).

Participants were considered sensitised to an allergen component in case of an ISU measurement of 0.3 or higher, based on the test manufacturer's recommendations.[21] Measurements were also reported as a detectable versus undetectable (lower detection limit was 0.06 ISU).

3.5 Microarray detection of N-glycan-specific IgE and IgG

Plasma IgE and IgG responses to 135 chemo-enzymatically synthesised N-glycans with and without core α-1,3-fucosylation and, or, β-1,2-xylosylation were assessed using a non-commercial microarray (**Figure S1, Research Papers 3 and 4).** The microarray slides were constructed by Dr Niels-Christian Reichardt's glycotechnology laboratory at Centro de Investigación Cooperativa en Biomateriales (CIC biomaGUNE), in San Sebastián, Spain. The microarray construction procedures have been published.[22,23]

Each microarray reaction site included fluorescently-labelled bovine serum albumin (BSA) as a printing control. The antibody binding assay was adapted from existing procedures,[24-27] as follows: Nexterion H N-hydroxysuccinimide-coated microarray slides (Schott AG, Mainz, Germany) (pre-blocked for 1 hour with 50mM ethanolamine in 50mM sodium borate buffer pH 9.0, and stored at -20°C) were thawed at room temperature (RT) and covered with silicone gaskets to create seven wells with printed microarrays per slide. Each microarray was incubated with 300 µl of a 1:30 plasma dilution in 1% BSA - 0.01% Tween20 for one hour at RT while shaking. After sequential washes with PBS-0.05% Tween20 and PBS, the slides were incubated for 30 minutes at RT in the dark with PromoFluor 647-labelled anti-human IgE (diluted 1/150 in PBS-0.01% Tween20) and Cy3-labelled anti-human IgG (diluted 1/1000 in PBS-0.01% Tween20), while shaking. After a final wash with PBS-0.05% Tween20, PBS and deionised water, sequentially, the slides were dried and kept in the dark until scanning. The slides were scanned for fluorescence at a 10µm resolution with a G2565BA scanner (Agilent Technologies, CA, USA) using 633nm and 532nm lasers for detection of reactivity to glycan-specific IgE and IgG, respectively.

3.5.1 Glycan microarray image processing and analysis of data

Using GenePix Pro 7.0 software (Molecular Devices, CA, USA), a spot-finding algorithm was used to align and re-size fluorescence spots in the microarray images, without setting a composite pixel intensity threshold. Data on median fluorescence intensity (MFI) for each spot and the local background were then exported to Microsoft Excel software. In all analyses, MFIs that were highlighted as artefacts by the GenePix Pro 7.0 software were excluded. Further processing of IgG MFIs in Excel was done based on procedures described by Oyelaran *et al.*[28] as follows: for each spot, the MFI of the spot was subtracted from the local background MFI. For each glycan structure, the average over four spots (or fewer, in case of unreliable data that were excluded) was obtained. Any MFI values below 150 were set to 150 to reduce noise at the low end of the MFI

range. Median values of negative controls included on each array were subtracted, and any negative values set to zero. Datasets were then log_2-transformed to normalize the data. Further processing of IgE MFIs in Excel was done as described by Amoah *et al.,*[24] as follows: for each IgE spot, the ratio of the MFI of the spot to the local background MFI was obtained and then multiplied by the average of background MFI for all the spots on the array. For each of the structures, the average over four spots (or less, in case of unreliable data that were excluded) was then log_2-transformed.

3.6 Other experimental methods

3.6.1 Allergen-specific IgG4 ELISA

All but the first 2 columns of MICROLON® high binding 96-well plates (Greiner bio-one, UK) were coated with 50µl of *D. pteronyssinus* or *B. germanica* allergens (Greer Labs, Lenoir, NC, USA) at a concentration of 5µg/ml in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer (0.1M, pH 9.6). Two-fold dilutions of human IgG4 (Sigma-Aldrich) standard, diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4ºC. Plates were washed with phosphate-buffered saline (PBS)-tween 20 solution, blocked with 150µl of skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4ºC with 50µl of plasma samples diluted 1/40 with 10% fetal bovine serum in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates overnight at 4ºC with 0.5µg/ml of biotinylated monoclonal mouse anti-human IgG4 (BD Pharmingen™), followed by a one-hour incubation with a streptavidin-Horseradish Peroxidase (strep-HRP) conjugate (Mast Group Ltd, Bootle, UK), diluted 1/3000 with assay buffer, at RT. Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped with 30µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgG4 concentrations (ng/ml) were interpolated from standard curves using

a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

3.6.2 Total IgE ELISA

96-well Maxisorp plates (VWR, U.S.A) were coated with 100µl of polyclonal rabbit anti human IgE (Agilent technologies, Dako, Denmark) diluted 1/1000 in bicarbonate buffer (0.1M, pH 9.6) and incubated overnight at 4ºC. Plates were then washed with phosphate-buffered saline (PBS)-tween 20 solution and blocked with 120µl of PBS-bovine serum albumin (BSA) solution for 1 hour at room temperature (RT). Plasma samples (100µl) diluted 1/50 in assay buffer (0.1M Tris pH 7.5 + 0.05% Tween-20), the blank (assay buffer) and National Institute for Biological Standards and Control (NIBSC) international IgE standards were added to the plates and incubated for 1 hour at RT. Plates were then washed and incubated with 100µl of biotinylated goat anti-human IgE (Vector laboratories, U.S.A, 0.5mg/ml), diluted 1/1000 with assay buffer, for 1 hour at RT. After another washing step, the plates were incubated with 100µl of streptavidin alkaline phosphatase (Roche Life Science), diluted 1/3000 with assay buffer, for 30 minutes at RT. 4-nitrophenyl phosphate disodium salt hexahydrate (p-NPP), diluted in diethanolamine buffer (DEA, 0.1M), was added, followed by 20 minutes incubation at RT in the dark for development. Sodium hydroxide (3M, 100µl) was then added to stop the reaction. Plates were read at 405 nm using an ELISA reader. Results were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

3.6.3 Total IgG4 ELISA

96-well Maxisorp plates (VWR, U.S.A) were coated with purified mouse anti-human IgG4 (BD Pharmingen™) in bicarbonate buffer (0.1 M, pH 9.6) overnight at 4ºC, and blocked for one hour with 3% skimmed milk in 1X PBS at room temperature (RT). Plates were then incubated with plasma samples (diluted 1/800 in 0.1M Tris pH 7.5 + 0.05% Tween 20) and IgG4 standards (Sigma Aldrich) for one hour. Antibody binding was detected by

incubating the plates with mouse anti-human IgG4 conjugated to horseradish peroxidase (Invitrogen) for one hour, followed by a colour reaction with o-phenylenediamine (Sigma Aldrich). Reactions were stopped with 2M Sulphuric acid. Absorbance was measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgG4 concentrations (ng/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

3.6.4 S. mansoni adult worm (SWA)- and egg (SEA)-specific IgE and IgG4 ELISA

All but the first 2 columns of 4HBX Immulon (Thermo Scientific, NY, USA) 96-well plates were coated with 50µl of SWA [8 µg/ml] or SEA [2.4 µg/ml] (purchased from Professor Michael J Doenhoff, University of Nottingham) in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer (0.1M, pH 9.6). Two-fold dilutions of human IgE (Calbiochem, Beeston, UK) or IgG4 (Sigma-Aldrich) standard, diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4^0C. Plates were washed with phosphate-buffered saline (PBS 1X)-tween 20 solution, blocked with 150µl of 1% skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4ºC with 50µl of plasma samples diluted 1/20 (IgE assay) or 1/200 (IgG4 assay) with 0.1% skimmed milk in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates overnight at 4ºC with 0.5µg/ml of biotinylated monoclonal mouse anti-human IgE or IgG4 (BD Pharmingen™). This was followed by a one-hour incubation with a streptavidin-Horseradish Peroxidase (strep-HRP) conjugate (Mast Group Ltd, Bootle, UK), diluted 1/4000 with assay buffer, at RT. Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped after 30 minutes with 25µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgE or IgG4 concentrations (ng/ml)

were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

3.6.5 S. mansoni adult worm (SWA)- and egg (SEA)-specific IgG ELISA

All but the first 2 columns of 4HX Immulon (VWR, UK, Cat No 735-0465) 96-well plates were coated with 50µl of SWA [8 µg/ml] or SEA [2.4 µg/ml] (purchased from Professor Michael J Doenhoff, University of Nottingham) in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer (0.1M, pH 9.6). Two-fold dilutions of human IgG (Sigma-Aldrich), diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4ºC. Plates were washed with phosphate-buffered saline (PBS)-Tween 20 solution, blocked with 150µl of 1% skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4ºC with 50µl of plasma samples diluted 1/3000 with 0.1% skimmed milk in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates for 1 hour at RT with 0.5µg/ml of polyclonal rabbit anti- human IgG/HRP (Dako, Denmark). Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped after 30 minutes with 25µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgG concentrations (ng/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

3.7 Selection of samples for antibody studies

The flow chart for selection of samples for antibody studies is shown in **Figure 3.3**, below.

In the rural (LaVIISWA outcome) survey, 2961 participants from the 26 study villages had a plasma sample stored and hence qualified to be selected for the ImmunoCAP IgE test. This test could not be conducted in all participants for cost reasons. Therefore, 780

participants (30 per village) were randomly selected using Stata 13.1 software (StataCorp, College Station, Texas, U.S.A). The main LaVIISWA trial analysis aimed to compare outcomes between the two trial arms.[1,3] A sample size of 780 was expected to give 80% power to detect a 35% relative difference in the prevalence of ImmunoCAP allergen-specific IgE sensitisation between the two trial arms, based on an assumed overall allergen-specific IgE sensitisation prevalence of 50% (from results at baseline), and an estimated coefficient of variation (in IgE levels between clusters) of 0.2. Enzyme-linked Immunosorbent Assays (ELISAs) for measurement of total, SWA-, SEA- and allergen-specific IgE and IgG4, and SWA- and SEA-specific IgG were conducted using the same 780 samples.

Samples for the glycan and ISAC microarray experiments were randomly selected from among those with ImmunoCAP data. The aim was to assay 50 samples per trial arm (100 in total). A total of 209 (glycan array) and 126 samples (ISAC array experiments, respectively), equally distributed across trial arms, were eventually tested, owing to availability of further funding from the Royal Society of Tropical Medicine and Hygiene (RSTMH) and the European Academy of Allergy and Clinical Immunology (EAACI). Although the sample size was arbitrary, and largely determined by available resources, similar numbers have been shown to be useful in published studies that have assessed differences in microarray-assessed antibody levels between helminth infected and uninfected, and allergic and non-allergic individuals,[24,29] and between children and adults.[25]

In the urban survey, 1356 participants had a plasma sample stored. Of these samples, 1345 had sufficient volume for antibody assays. All of these plasma samples were assessed by ELISA for total, SWA-, SEA- and allergen-specific IgE and IgG4, and SWA- and SEA-specific IgG. For reasons of cost, the ImmunoCAP IgE test could not be conducted in all these samples. ImmunoCAP data on cockroach- and dust mite-specific IgE were available for 780 rural survey participants, hence calculations could be

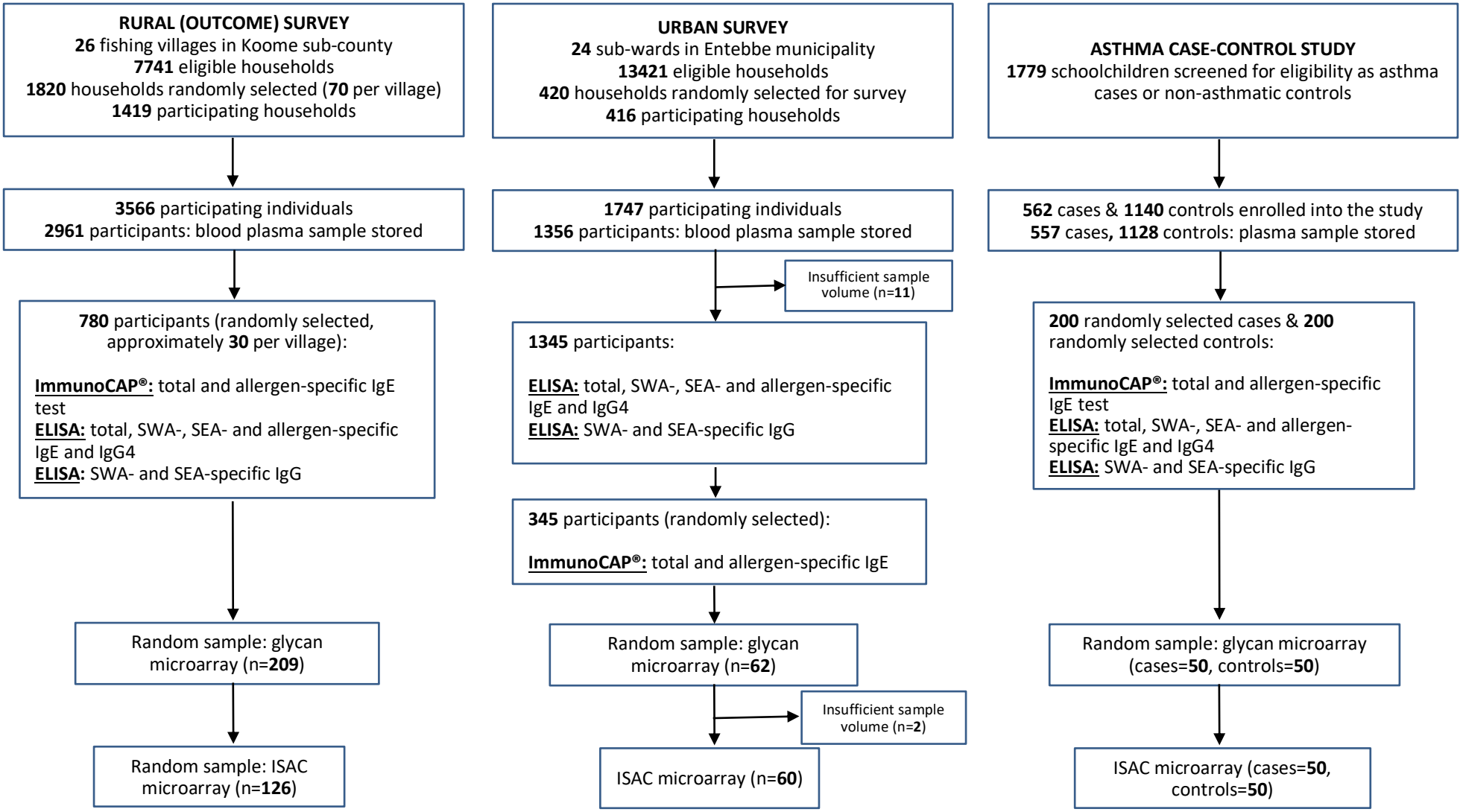
conducted to estimate how many urban survey plasma samples were required to attain significant differences in the prevalence of ImmunoCAP positivity between the urban and rural survey. Assuming a 35% prevalence of ImmunoCAP positivity to either cockroach or dust mite in the rural survey, 80% power, 5% significance level, and a design effect of 1.3, 353 urban survey participants were estimated to be enough to detect an absolute difference in proportion positive of 0.10 between the rural and urban survey. The design effect was considered in the sample size calculations because data from both the rural and the urban survey were clustered (rural survey by village and household, urban survey by sampling area and household). By definition, the design effect is the ratio of the variance of estimates calculated from a cluster survey to the variance of estimates calculated under simple random sampling. Therefore, 353 plasma samples were randomly selected (using a Stata program) for the ImmunoCAP test; however, only 345 of these samples were remaining with sufficient volume at the time of the assay and could be assessed.

Urban survey samples for the glycan and ISAC microarray experiments were randomly selected from among those with ImmunoCAP data. The main aim was to conduct rural-urban comparisons of antibody profiles. A sample size of 50 was deemed sufficient, based on previous studies doing similar comparisons.[24] Eventually, the actual numbers of plasma samples that were assayed were 62 and 60 for the glycan and ISAC microarrays, respectively, to avoid wastage of unused microarray chips.

In the asthma case-control study, 557 cases and 1128 controls had a plasma sample stored. For cost reasons, 400 participants (200 asthmatics and 200 controls) were randomly selected using Stata software. This number had 90% power to detect a significant positive association (at an odds ratio of 2) between asthma and ImmunoCAP positivity to any of dust mite, cockroach or peanut (IgE ≥0.35 kU/L), based on 5% significance level and an assumed prevalence of ImmunoCAP positivity of 55% among non-asthmatics (based on results from the urban survey). ELISA-based antibody studies

were conducted using the same samples. For glycan and ISAC microarray experiments, a sample size of 50 cases and 50 controls was deemed sufficient, based on numbers used in the rural and the urban survey.

Figure 3.3. Flow chart for selection of samples for antibody studies



3.8 Data analysis

Quantitative and qualitative data used in this PhD project were generated from questionnaires, standard clinical record forms (including clinical measurements and field laboratory measurements, such as parasite counts) and immunological assays. These data were entered using Microsoft Access and Excel. Information from questionnaires was also captured on ultra-mobile personal computers (UMPCs) and exported to database servers in Microsoft Access format. The data were then exported to Stata version 13.1 (StataCorp, College Station, Texas, USA), GraphPad Prism versions 6.0e and 7.0a (Fay Avenue, La Jolla, CA, USA) and R software (R foundation for Statistical Computing, Vienna, Austria) via the RStudio interface (version 1.1.383, RStudio, Inc. Boston, USA), for statistical analysis.

Analysis of data generated during this PhD project is detailed in the Research Paper chapters in this thesis (**Chapters 4 – 7**) and in **Appendix 3** (Research Paper 7). This section describes 1) approaches that were used for analysis of data while accounting for the cluster survey design of the rural and urban surveys, 2) how normal and skewed immunological data were handled, 3) correction for multiple testing and 4) techniques for reduction and subsequent analysis of antibody microarray data.

3.8.1 Adjusting for survey design in the rural surveys and the urban survey

The rural surveys were characterised by village-level cluster randomisation.[1] For the outcome survey, initial analyses compared antibody profiles between standard and intensive trial intervention, using statistical methods that allowed for within-cluster correlations. Primary analyses were done at the cluster level; cluster-specific proportions (for binary outcomes) and means (for quantitative outcomes) were calculated and then compared between trial arms using t-tests. Adjusted analyses were performed to account for confounders that might not have been balanced by the randomisation procedures.

As already mentioned, intensive versus standard treatment reduced hookworm prevalence

and *S. mansoni* infection intensity, but it had no effect on the overall prevalence of other nematodes, *S. mansoni,* allergy-related outcomes (**Appendix 3**) or on most laboratory outcomes (this thesis). Therefore, several chapters in this thesis presenting results from the 'rural survey' have used data combined from both trial arms of the LaVIISWA outcome survey. These analyses were primarily based on individual-level data and were conducted using "svy" survey commands in Stata to allow for the clustering of respondents within households and households within clusters. Sampling weights were calculated from the number of households in each cluster, to reflect the fact that households in smaller villages had a higher probability of selection than households in larger villages (since an equal number of households was selected from each village). These sampling weights were also employed in the analysis using the svy commands.

In the urban survey, analysis methods accounted for clustering of respondents within households and households within sub-wards. The number of geographical segments selected for sampling was proportional to the population size of each sub-ward, hence the study design was considered self-weighting. Therefore, svy commands were used to allow for clustering within sub-wards but not for weighting.

3.8.2 Distribution of antibody data

Similar to most data generated from immunological assays,[30] antibody data were predominantly right-skewed. Log transformations were applied to normalize the data. In some instances, the data maintained a skewed distribution after log transformation. The bootstrap resampling method[31] is useful in analysis of skewed data without making any parametric assumptions; however, it is incompatible with svy commands in Stata and hence was not used in analysis of data in this project. Instead, where possible, non-parametric statistical tests (such as the Mann Whitney U test and the Kruskal-Wallis test) were employed. However, these tests cannot be used for multivariable analyses, hence

regression analyses that assume parametric distributions were used. Of note, use of parametric tests to analyse skewed data may still provide approximately valid test statistics, provided sample sizes are large enough and the data are not severely skewed.[30]

3.8.3 Correction for multiple testing

Many of the microarray-determined anti-carbohydrate and anti-allergen antibody responses (**Chapter 6** and **Chapter 7**) were strongly correlated. The commonly used Bonferroni correction for multiple testing would be a conservative approach to use with these data, because it assumes independence between tests and is prone to type II errors when the sample size is modest.[32,33] Therefore, using Stata software, a Monte Carlo simulation approach[34] based on 1000 permutations was adopted, to generate empirical p-values. An example of a Stata 'do file' used to perform permutation testing is shown in **Appendix 5**.

Chapters 4 and **5** report a large number of statistical tests for associations between epidemiological / immunological outcomes and helminth infections and allergy-related outcomes. Here, multiplicity was not formally adjusted for; instead, the focus was on patterns of association and consistency of results, and on biological plausibility with reference to other published findings.

3.8.4 Anti-glycan antibody responses: data reduction procedures

Principal component analysis (PCA) was run in Stata as a data reduction technique to transform groups of correlated anti-glycan responses into fewer, uncorrelated artificial variables (principal components, PCs). Crude and adjusted associations between PC scores and various comparison groups were assessed using survey design-based linear regression. Another data reduction technique was hierarchical clustering analysis (HCA, complete linkage using Euclidean distance), conducted in R to identify homogeneous sets of N-glycan-specific responses. The resultant response clusters were assessed for

associations with comparison groups of interest using the global test[35,36] executed in R using the Globaltest package (version 5.33.0).

3.9 Ethical approvals

The LaVIISWA trial, the urban survey and the asthma case-control study obtained ethical approval from ethics committees of Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, and Uganda National Council for Science and Technology. To conduct my PhD research using samples collected from these studies, I obtained approval from the ethics committee of London School of Hygiene and Tropical Medicine. In all three studies, participants provided signed informed consent. For minors, participants' parents or guardians gave this consent. Children aged eight years and above additionally provided signed informed assent. Consent and assent forms were translated into the local language (Luganda), for participants to choose which language they preferred or understood better. Copies of English language versions of ethical approvals are included in **Appendix 4**.

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CHAPTER 4. URBAN-RURAL DIFFERENCES IN RISK FACTORS FOR ALLERGY-RELATED OUTCOMES IN UGANDA: A ROLE FOR HELMINTHS?

4.1 Preamble

This chapter uses questionnaire, clinical and laboratory data from the LaVIISWA outcome survey (hereinafter "rural survey") and the urban survey (both described in Chapter 3) to investigate the extent to which helminth exposure influences rural-urban differences in prevalence of allergy-related outcomes, and in epidemiological and immunological risk factors for these outcomes (**thesis objective 1**). Results are presented in Research paper 1, which is titled "Do helminth infections underpin urban-rural differences in risk factors for allergy-related outcomes?", and which has been published as an original article in *Clinical and Experimental Allergy.*

Urban-rural differences in 1) prevalence of allergy-related outcomes, helminth infections and helminth-specific antibody concentrations, and 2) epidemiological and immunological (schistosome-specific antibodies) risk factors for allergy-related outcomes, are shown. Importantly, this chapter assesses whether helminth (*S. mansoni*) infections are effect modifiers of associations between the above-mentioned risk factors and allergy-related outcomes, and whether they were likely to mediate the differences seen between the urban and the rural settings. These analyses lay the foundation for the overarching aim of this thesis, which is to understand how helminth- and allergen-specific antibody profiles relate to epidemiological trends of allergy in rural and urban Uganda (assessed in more detail in the next chapters).

4.2 Research paper 1: Do helminth infections underpin urban-rural differences in risk factors for allergy-related outcomes?

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SECTION A – Student Details

Student	GYAVIIRA NKURUNUNGI
Principal Supervisor	ALISON ELLIOTT
Thesis Title	HELMINTH-ALLERGY ASSOCIATIONS IN RURAL AND URBAN UGANDA: INSIGHTS FROM ANTIBODY STUDIES

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	CLINICAL AND EXPERIMENTAL ALLERGY		
When was the work published?	11th January 2019		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I participated in the design of the laboratory studies and performed the experiments. I participated in cleaning of epidemiological data and analysed the results from both laboratory and epidemiological investigations. I wrote the manuscript.

Student Signature: [redacted] Date: 14th FEB 2019

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ORIGINAL ARTICLE

Epidemiology of Allergic Disease

Do helminth infections underpin urban-rural differences in risk factors for allergy-related outcomes?

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Summary

Background: It is proposed that helminth exposure protects against allergy-related disease, by mechanisms that include disconnecting risk factors (such as atopy) from effector responses.

Objective: We aimed to assess how helminth exposure influences rural-urban differences in risk factors for allergy-related outcomes in tropical low- and middle-income countries.

Methods: In cross-sectional surveys in Ugandan rural *Schistosoma mansoni* (*Sm*)-endemic islands, and in nearby mainland urban communities with lower helminth exposure, we assessed risk factors for atopy (allergen-specific skin prick test [SPT] reactivity and IgE [asIgE] sensitization) and clinical allergy-related outcomes (wheeze, urticaria, rhinitis and visible flexural dermatitis), and effect modification by *Sm* exposure.

Results: Dermatitis and SPT reactivity were more prevalent among urban participants, urticaria and asIgE sensitization among rural participants. Pairwise associations between clinical outcomes, and between atopy and clinical outcomes, were stronger in the urban survey. In the rural survey, SPT positivity was inversely associated with bathing in lakewater, *Schistosoma*-specific IgG4 and *Sm* infection. In the urban survey, SPT positivity was positively associated with age, non-Ugandan maternal tribe, being born in a city/town, BCG scar and light *Sm* infection. Setting (rural vs urban) was an effect modifier for risk factors including *Sm*- and *Schistosoma*-specific IgG4. In both surveys, the dominant risk factors for asIgE sensitization were *Schistosoma*-specific antibody levels and helminth infections. Handwashing and recent malaria treatment reduced odds of asIgE sensitization among rural but not urban participants. Risk factors for clinical outcomes also differed by setting. Despite suggestive

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trends, we did not find sufficient evidence to conclude that helminth (*Sm*) exposure explained rural-urban differences in risk factors.

Conclusions and clinical relevance: Risk factors for allergy-related outcomes differ between rural and urban communities in Uganda but helminth exposure is unlikely to be the sole mechanism of the observed effect modification between the two settings. Other environmental exposures may contribute significantly.

KEYWORDS
allergy, effect modification, helminths, risk factors, Uganda, urban-rural

Advances in health and hygiene practices have transformed high-income countries into "cleaner" environments, with reduced infection exposure. Consequently, homeostatic immunomodulatory effects of exposure to microbes and parasites that co-evolved with mammalian species (the "old friends hypothesis") have been lost.[1] The surge in allergy-related diseases alongside other chronic inflammatory diseases in high-income countries over recent decades has been partly attributed to this phenomenon.[2] Although other environmental exposures[3] may contribute, substantial support for the "old friends hypothesis" comes from studies in high-income countries,[4-9] which show that traditional farming and related microbial exposures[10] are associated with protection against allergy-related diseases. Additional evidence suggests a parallel relationship between ongoing urbanization and increasing allergy-related disease prevalence in tropical low- and middle-income countries (LMICs).[11,12]

Akin to farming environments in high-income countries, rural LMIC settings are relatively protected against allergy-related diseases.[13-17] Animal models and in vitro experiments in human samples have identified helminths as potent inhibitors of allergic reactions,[18-20] leading to the hypothesis that they are partly responsible for the low overall prevalence of allergy-related diseases in tropical LMICs and the observed rural-urban disparities in allergy-related disease prevalence in the same settings.[16,21] Helminths may dissociate risk factors, such as atopy, from allergy-related disease: work in Ugandan children showing that hookworm infection dissociates allergen-specific IgE from the effector phase of the allergic response[22] is strongly suggestive. However, little comparative analysis of risk factors for allergy in rural vs urban LMIC settings has been conducted. Exploration of these factors in LMICs, where an epidemiological transition is ongoing, provides an unprecedented opportunity to better understand interactions between the environment and the allergic pathway and allergy-related disease outcomes.

Using data generated from two surveys in Uganda, one in rural helminth-endemic Lake Victoria island fishing villages and another in nearby mainland urban communities with lower helminth exposure, we investigated socio-demographic, behavioural, clinical and immunological characteristics as risk factors for allergy-related outcomes and assessed whether helminth infections contribute to rural-urban differences in these risk factors.

2 | METHODS

2.1 | Study settings and procedures

Rural participants were residents of 26 helminth-endemic fishing villages of Koome islands, Mukono district, Uganda (population 18 778 in 2014[23]). Urban participants were residents of Entebbe Municipality, a lower helminth exposure area situated on the northern shores of Lake Victoria, 40 km southwest of the Ugandan capital, Kampala, and 35 km from Koome. The municipality had approximately 69 430 inhabitants in 2014,[23] distributed across 24 sub-wards, the smallest administrative units.

The "rural survey" was part of the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA; ISRCTN47196031), a cluster-randomized trial of standard vs intensive anthelminthic intervention, described elsewhere.[24,25] A baseline household survey preceded the trial intervention; helminth-allergy associations at baseline have been reported.[24] A household-based

allergy outcomes survey (the "rural survey") was conducted between September 2015 and August 2016, following 3 years of anthelminthic intervention: there was no difference in the prevalence of allergy outcomes between the two trial arms.[26] Sampling for the survey involved random selection of 70 households from each village using a Stata program. All household members (1 year and older) of selected households were then invited to participate. Permission for household participation was granted by the household head.

The urban survey of allergy-related outcomes (September 2016–September 2017) was designed intentionally to collect data from Entebbe municipality for comparison with the helminth-endemic rural survey. Before the start of the survey, each sub-ward was mapped onto satellite imagery of the municipality. A random point generation function of ArcGIS software (version 10.4.1, Environmental Systems Research Institute, Redlands, CA) was then used to generate random starting points within each sub-ward. The number of starting points selected was proportional to the population size of the sub-ward. Coordinates of the random starting points generated were loaded onto geographic information system (GIS) devices (eTrex®, Garmin™ Ltd, Olathe, KS). These devices were then used in the field to identify the selected random points, from which the nearest four houses were surveyed.

There was no randomization to intensive or standard anthelminthic treatment in the urban survey; however, all other procedures were designed to be identical in both the urban and the rural survey.

Following written informed consent and assent, questionnaires were completed for each participant, capturing socio-demographic, clinical and behavioural characteristics as well as asthma, eczema and allergy symptoms. The latter employed questions based on the International Study on Allergy and Asthma in Children (ISAAC) questionnaire. Blood, stool and mid-stream urine were collected. Blood samples were used for haemo-parasitology, HIV serology and storage of plasma and cells for immunoassays. One stool sample per participant was examined for intestinal helminth infections using the Kato-Katz method[27] (two slides, read by different technologists). The remaining sample was stored and later investigated for *Schistosoma mansoni (Sm)*, *Strongyloides stercoralis* and hookworm (*Necator americanus*) infections using multiplex real-time PCR.[28,29] Urine was assessed for *Sm* circulating cathodic antigen (CCA, Rapid Medical Diagnostics, Pretoria, South Africa). *Schistosoma* egg [SEA]- and adult worm [SWA] antigen-specific immunoglobulin (Ig)E, IgG4 and IgG levels were assessed in plasma using in-house ELISAs (Data S1).

Ethics committees of Uganda Virus Research Institute (refs: GC/127/12/05/03 and GC/127/16/02/547) and London School of Hygiene and Tropical Medicine, (refs: 6187 and 10709) and the Uganda National Council for Science and Technology (ref: HS1183 and HS2036) approved both surveys.

2.2 | Allergy-related outcomes

Outcomes were skin prick test (SPT) reactivity to allergens common in our setting,[30] allergen-specific IgE (asIgE) sensitization, self-reported recent (previous 12 months) wheeze, recent rhinitis, recent urticarial rash and visible flexural dermatitis.

Skin prick test reactivity (wheal ≥3 mm diameter after 15 minutes in the presence of saline [negative] and histamine [positive] controls) to dust mites (*Dermatophagoides* mix, *Blomia tropicalis*) and German cockroach (*Blattella germanica*) (ALK-Abelló; supplied by Laboratory Specialities [Pty] Ltd., Randburg, South Africa) was

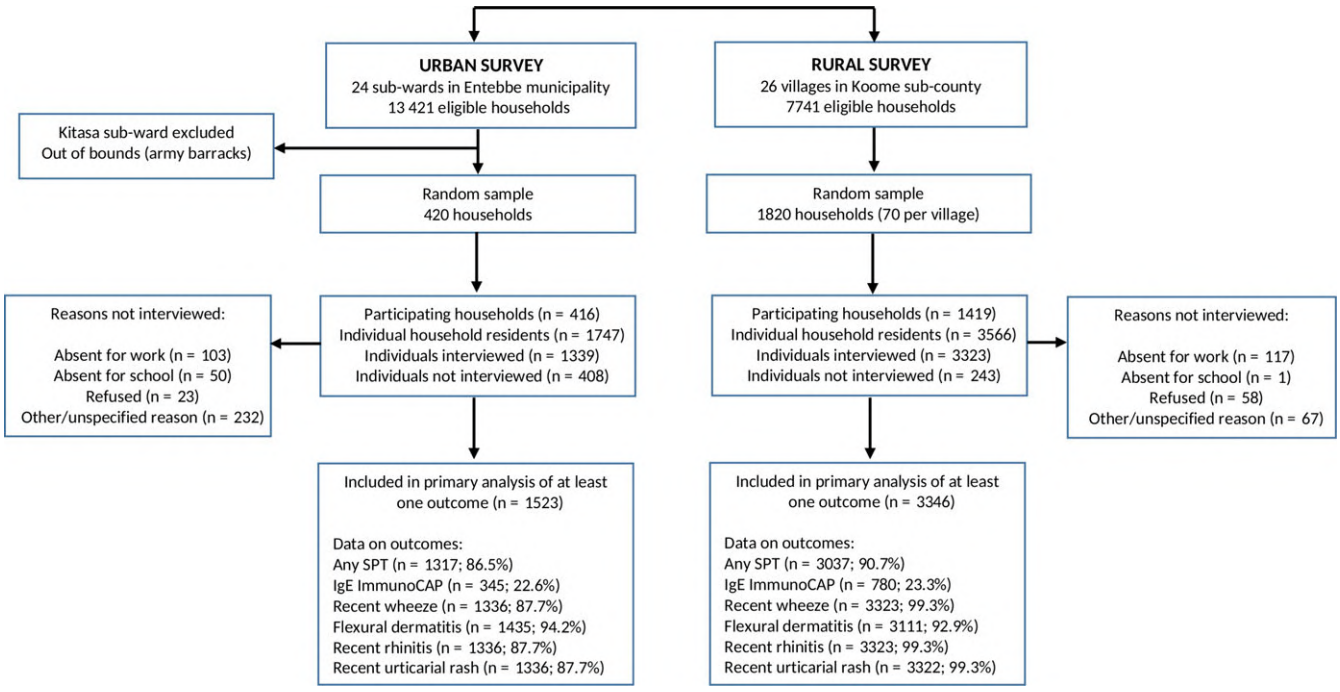

FIGURE 1 Study flowchart

assessed using standard procedures.[31] SPT reactivity was defined primarily as a positive response to any of the three allergens. SPT reactivity was also analysed as a positive vs negative response to individual allergens.

Whole allergen (*Dermatophagoides pteronyssinus*, peanut [*A hypogaea]* and *B germanica*) extract-specific plasma IgE (asIgE) was measured by ImmunoCAP® (ThermoFisher Scientific, Uppsala, Sweden) in a sample of 780 and 345 rural and urban survey participants, respectively, randomly selected from those with sufficient volume of stored plasma. Allergen-specific IgE sensitization was defined as a positive ImmunoCAP response (IgE concentration ≥0.35kU/L) to any of the three allergens and as a positive vs negative ImmunoCAP response for individual allergens. ImmunoCAP IgE outcomes were also analysed as continuous variables.

Wheeze is considered a good proxy for asthma in epidemiological studies[32] and was assessed separately in two age groups (≥5 years and <5 years) using an interviewer-administered ISAAC questionnaire. The principal age group of interest was ≥5 years because wheeze cannot be assumed to represent asthma in children below 5 years.[33]

Data on recent rhinitis (runny/blocked nose or sneezing accompanied by watery and itchy eyes, in the absence of cold or "flu") and urticarial rash (pruritic rash with weals, known as "ebilogologo" in the local language [Luganda]) were obtained by questionnaire. Visible flexural dermatitis was assessed (by staff trained on Williams' online manual[34]) as an erythematous rash with surface change in and around skin creases.[35,36]

2.3 | Statistical methods

Data analysis was conducted using Stata 13.1 (College Station, TX). The following were assessed as potential risk factors for allergy-related outcomes: socio-demographic characteristics (age, sex, presence of older/younger siblings, maternal tribe, paternal tribe, location of birth and occupation), behavioural characteristics (frequency of lake contact, type of bathing water, handwashing behaviour, footwear outside the house, smoking and alcohol use), clinical characteristics (helminth infections, exposure to anthelminthic treatment in utero, anthelminthic treatment in last 12 months, parental history of allergies, BCG scar, immunisation history, malaria treatment in last 12 months, malaria infection and HIV infection) and immunological characteristics (plasma SEA- and SWA-specific IgE, IgG4 and IgG levels). Additionally, allergy-related outcomes were independently assessed as risk factors for each other.

Stata "svy" commands were used to allow for clustering of participants within villages and for the non-self-weighting design of the rural survey[24] and for clustering by sub-ward in the urban survey.

Logistic regression was used to compare the prevalence of outcomes and other characteristics between the rural and urban survey and to assess associations between each pair of allergy-related outcomes in both surveys. Population attributable fractions (PAFs) for pairs of allergy-related outcomes were calculated. Interaction tests were done to assess whether these associations differed by setting. Unadjusted and adjusted odds ratios (OR) for associations between exposures and allergy-related outcomes were estimated using univariable and multivariable logistic regression. Additionally, linear regression was used in secondary analyses of ImmunoCAP IgE outcomes as continuous variables. Age, sex (a priori) and factors showing evidence of crude association with an outcome ($P < 0.05$) were considered in multivariable analyses for that outcome. We hypothesized that helminth infections might be key mediating factors on the causal pathway between urban/rural residence and allergy-related outcomes; hence, helminths (and *Sm*-specific antibody responses and other "helminth-related" factors such as frequency of lake contact and occupation) were not included in multivariable analyses for other risk factors. The potential mediating role of helminths was then investigated separately by assessing whether associations between non-helminth-related risk factors and allergy-related outcomes changed substantially when adjusted for *Sm* infections and *Schistosoma*-specific antibody levels. These analyses were initially conducted separately for each survey. Subsequently, we merged data from the two surveys and tested for interaction between the rural and urban survey, to assess whether risk factors for allergy outcomes differed by setting. Here, we also assessed the potential role of helminths in urban-rural interactions by comparing interaction *P* values before and after adjusting for *Sm* infection. A 5% significance level was used for all analyses.

3 | RESULTS

3.1 | Participants' characteristics

Flowcharts of the surveys are shown in Figure 1. Of 1820 households randomly selected for the rural survey (70 from each of the 26 villages), 1419 (78%) took part. There were 3566 individuals inhabiting the 1419 participating households; 3323 (93.2%) were interviewed and 3346 (93.8%) had data on at least one allergy-related outcome. Of 420 households randomly selected for the urban survey, 416 (99%) took part. There were 1747 individuals inhabiting the 416 households; 1339 (77%) were interviewed and 1523 (87%) had data on at least one allergy-related outcome.

Participant characteristics differed between the two study settings (Table 1). Significantly, rural, compared to urban participants, were more likely to be infected with helminths (including *Sm*), malaria and HIV, had higher median levels of *Schistosoma*-specific antibodies and were more likely to report anthelminthic or malaria treatment in the previous 12 months. Dermatitis and SPT reactivity were more prevalent among urban participants, while asIgE sensitization and urticaria were more common among rural participants (Table 1 and Figure 2A). The prevalence of wheeze and rhinitis was similar between the two communities.

3.2 | Associations between allergy-related outcomes

Crude associations between allergy-related outcomes are shown in Table 2. Individuals who were ImmunoCAP asIgE sensitized were

more likely to have a positive SPT response in both surveys; the PAF for SPT reactivity associated with asIgE sensitization was 86.1% and 80.9% for the urban and rural survey, respectively. Atopy measures (asIgE, SPT) were generally more strongly associated with other allergy-related conditions in the urban compared to rural survey; asIgE-rhinitis (interaction $P = 0.081$), asIgE-urticaria (interaction $P = 0.056$), SPT-rhinitis (interaction $P = 0.019$) and SPT-urticaria (interaction $P = 0.005$) associations approached statistical significance. Another major difference was that urticaria was associated with wheeze, rhinitis and SPT reactivity in the urban survey, but not with any allergy-related outcome in the rural survey.

We hypothesized that helminth infection, particularly *Sm* infection, might mediate this effect modification between the urban and rural setting (Figure 2B). However, the comparison of crude associations (reported above) with associations adjusted for current *Sm* infection (generally, or categorized by infection intensity) and *Schistosoma*-specific antibody concentrations did not show clear differences in the test statistics (Table S1); hence, any mediating role of current *Sm* infection, including effects on interactions between the rural and urban survey, was not evident.

3.3 | Factors associated with skin prick test reactivity

Table 3 and Table S2 show factors associated with SPT reactivity to any of *Dermatophagoides* mix, *B tropicalis* or *B germanica*. In the urban survey, increasing age, non-Ugandan maternal tribe, being born in a city (compared to town or village) and having a BCG scar were positively associated with SPT reactivity. Additionally, light *Sm* infection (KK) and *Sm* infection (PCR) were positively associated with SPT reactivity in the urban survey, in sharp contrast to observations in the rural survey, where current *Sm* infection (KK, PCR and CCA) was associated with reduced odds of SPT reactivity. This rural-urban difference was statistically significant (interaction P values = 0.002 and 0.015 for *Sm*-PCR and *Sm*-KK intensity, respectively). Other factors inversely associated with SPT reactivity in the rural survey were related to helminth infections and included bathing in lakewater and SWA-specific IgG4.

In addition to the *Sm*-SPT association, tests for interaction showed that associations between several other risk factors and SPT reactivity differed by survey setting. Being male ($P = 0.015$), maternal history of allergies ($P = 0.013$), SWA-specific IgG4 ($P = 0.011$) and hand washing ($P = 0.001$) were positively associated with SPT in the urban survey but inversely associated with the same outcome in the rural survey. The inverse association between SPT and being born in a village (compared to town or city) was stronger in the urban compared to rural survey ($P = 0.041$).

Associations with SPT reactivity to individual allergens are summarized in Table S3, and paint a similar picture.

Comparison of models with and without additional adjustment for current *Sm* infection (generally, or categorized by infection intensity) and *Schistosoma*-specific antibodies did not suggest any mediating role of *Sm* infection in associations between non-helminth-related risk factors and SPT reactivity, or in interactions between the rural and urban survey (Table S4A).

3.4 | Factors associated with allergen-specific IgE sensitization

Table 4 and Table S5 show factors associated with ImmunoCAP IgE sensitization to any of *D pteronyssinus*, *A hypogaea* or *B germanica* extracts. In the urban survey, the presence of younger siblings and SWA-specific IgG were associated with asIgE sensitization. Rural participants who washed hands after toilet use, slept under a mosquito net and/or had recently been treated for malaria were less likely to be asIgE sensitized. Engaging in agricultural/fishing/lake-related activities or being unemployed, *Sm* infection (KK) and intensity, and elevated SWA-specific IgE increased the odds of asIgE sensitization.

The presence of younger siblings (interaction $P = 0.008$) and hand washing (interaction $P = 0.003$) were associated with reduced odds of asIgE sensitization in the rural but not the urban survey (Table 4). Adjusting for *Sm* infection in multivariable analysis models did not suggest a mediating role for *Sm* in these rural-urban differences (Table S4B).

Table S6 summarizes factors associated with ImmunoCAP asIgE sensitization to individual allergens: *Schistosoma*-specific antibody levels and helminth infections were the predominant risk factors in both surveys. Hygiene practices (washing and bathing) reduced the odds of sensitization in the rural but not urban survey.

3.5 | Factors associated with clinical allergy-related outcomes

Factors associated with self-reported recent wheeze, urticarial rash and rhinitis are shown in Table S7. Risk factors for visible flexural dermatitis could not be assessed because it was rare in both settings. In the urban survey, the presence of older siblings, handwashing before eating, SWA-specific IgG and SEA-specific IgG were inversely associated with wheezing. In the rural survey, female sex and presence of any nematode infection were inversely associated with wheezing, while increasing age, SWA-specific IgG, SEA-specific IgG and paternal history of allergies increased the odds of wheezing. Non-Ugandan paternal tribe (interaction $P < 0.001$) increased the odds of wheezing in the urban but not rural survey, while SWA-specific IgG ($P < 0.001$) and SEA-specific IgG ($P = 0.001$) were positively associated with wheezing in the rural but not the urban survey.

Urban individuals who received any anthelminthic treatment in the previous 12 months were more likely to report urticarial rash. In the rural survey, increasing age, maternal history of allergies, SEA-specific IgE and recent malaria treatment were associated with urticaria. The association between SEA-specific IgE and urticaria was positive in the rural but not urban survey (interaction $P = 0.022$). No other significant interactions were observed.

Maternal and paternal history of allergies, and HIV infection were associated with rhinitis in the urban survey. The following were risk factors for rhinitis in the rural survey: increasing age, presence of

TABLE 1 Characteristics of study participants

Characteristics	Urban survey n/N (%)[a]	Rural survey n/N (%)[a]	*P* value*
Socio-demographic			
Age in (y), median (IQR)	20 (8, 31)	24 (8, 34)	0.329**
Male sex	688/1610 (42.7)	**1738/3350 (49.5)**	0.002
Place of birth			
City	**53/513 (10.3)**	61/2406 (2.9)	
Town	**138/513 (26.9)**	254/2406 (10.4)	
Village	322/513 (62.7)	**2091/2406 (86.7)**	<0.001
Maternal tribe, larger region grouping			
Central Uganda	605/1331 (45.5)	1197/3304 (36.5)	
Other, Ugandan	607/1331 (45.6)	1588/3304 (48.1)	
Non-Ugandan, African	119/1331 (8.9)	519/3304 (15.4)	0.020
Paternal tribe, larger region grouping			
Central Uganda	593/1334 (44.5)	1343/3317 (39.8)	
Other, Ugandan	624/1334 (46.8)	1556/3317 (47.5)	
Non-Ugandan, African	117/1334 (8.7)	418/3317 (12.7)	0.208
Maternal history of allergies (general)	93/1187 (7.8)	**366/2930 (12.7)**	<0.001
Paternal history of allergies (general)	30/1117 (2.6)	**171/2796 (5.7)**	0.005
Maternal history of asthma	27/1266 (2.1)	93/2931 (3.4)	0.167
Paternal history of asthma	27/1218 (2.2)	62/2796 (2.3)	0.950
Maternal history of eczema	35/1229 (2.8)	131/2931 (4.5)	0.206
Paternal history of eczema	15/1159 (1.3)	**96/2795 (2.9)**	0.028
Occupation, grouped by type			
Student or child (not at school)	**662/1338 (49.5)**	1166/3323 (36.7)	
Unemployed or housewife	**292/1338 (21.8)**	301/3323 (8.7)	
Agricultural, fishing or lake related	60/1338 (4.5)	**1389/3323 (38.8)**	
Professional or service providers (Shops, saloons, bars, restaurants, entertainment)	**324/1338 (24.2)**	467/3323 (15.6)	<0.001
Helminth infections			
S mansoni (KK)	86/1197 (7.2)	**846/2751 (31.8)**	<0.001
S mansoni intensity (KK)			
Uninfected	**1111/1197 (92.8)**	1905/2751 (68.2)	
Low	41/1197 (3.4)	**425/2751 (15.7)**	
Moderate	31/1197 (2.6)	**231/2751 (9.1)**	
Heavy	14/1197 (1.1)	**190/2751 (7.1)**	<0.001
S mansoni (urine CCA)	581/1318 (44.1)	**2445/2879 (85.6)**	<0.001
S mansoni (PCR)	204/1191 (17.1)	**1338/2747 (50.0)**	<0.001
A lumbricoides (KK)	0/1197 (0.0)	14/2751 (0.4)	
Trichuris trichiura (KK)	21/1196 (1.8)	**245/2751 (7.8)**	<0.001
N americanus (PCR)	56/1191 (4.7)	**259/2747 (8.4)**	0.016
S stercoralis (PCR)	29/1191 (2.4)	**190/2747 (6.2)**	<0.001
Schistosoma-specific antibody levels			
SEA-specific IgE (μg/mL), median (IQR)	2.7 (2.6, 2.8)	**4.6 (4.3, 4.8)**	<0.001**
SWA-specific IgE (μg/mL), median (IQR)	2.2 (2.1, 2.4)	**4.9 (4.6, 5.1)**	<0.001**
SEA-specific IgG4 (μg/mL), median (IQR)	30.8 (27.8, 37.3)	**278.6 (228.7, 322.4)**	<0.001**
SWA-specific IgG4 (μg/mL), median (IQR)	42.7 (40.5, 44.1)	**108.6 (98.3, 124.7)**	<0.001**

(Continues)

TABLE 1 (Continued)

Characteristics	Urban survey n/N (%)[a]	Rural survey n/N (%)[a]	*P* value*
SEA-specific IgG (μg/mL), median (IQR)	777.9 (744.6, 806.1)	**1975.4 (1848.0, 2096.4)**	<0.001**
SWA-specific IgG (μg/mL), median (IQR)	795.4 (771.2, 828.6)	**1497.2 (1429.4, 1561.5)**	<0.001**
Allergy-related outcomes			
Skin prick test reactivity			
Any	**302/1317 (22.9)**	576/3037 (19.1)	0.054
Dermatophagoides mix	**228/1317 (17.3)**	326/3037 (10.5)	<0.001
B tropicalis	**184/1317 (13.9)**	229/3036 (7.9)	<0.001
B germanica	186/1320 (14.1)	350/3035 (11.8)	0.137
Allergen-specific IgE (≥0.35 kU/L, ImmunoCAP)			
Any	148/345 (42.9)	**437/780 (55.1)**	0.007
D pteronyssinus	104/345 (30.1)	264/780 (33.2)	0.421
B germanica	118/345 (34.2)	**393/780 (49.8)**	<0.001
A hypogaea	41/345 (11.8)	114/780 (14.9)	0.266
Total IgE (kU/L), median (IQR)	159 (56, 522)	**672 (249, 1942)**	<0.001
Wheeze in last 12 mo, age<5 y	3/229 (1.3)	9/547 (1.4)	0.972
Wheeze in last 12 mo, age ≥ 5 y	24/1107 (2.2)	87/2776 (3.2)	0.190
Visible flexural dermatitis	**22/1435 (1.5)**	5/3111 (0.1)	<0.001
Rhinitis in last 12 mo	45/1336 (3.4)	104/3323 (3.2)	0.806
Urticarial rash in last 12 mo	53/1336 (3.9)	**334/3322 (9.9)**	<0.001
Other			
Any worm treatment in the last 12 mo	795/1296 (61.3)	**2938/3307 (87.7)**	<0.001
Malaria treatment in the last 12 mos	506/1336 (37.8)	**1993/3323 (60.8)**	<0.001
P falciparum positivity by blood smear	3/1347 (0.2)	**102/2923 (3.7)**	<0.001
HIV infection	66/1339 (4.9)	**402/2399 (17.3)**	<0.001

CCA: circulating cathodic antigen; IQR: interquartile range; KK: Kato-Katz; PCR: polymerase chain reaction; SEA: Schistosoma egg antigen; SWA: Schistosoma adult worm antigen.

[a]Percentages adjusted for survey design. Percentages that are significantly higher in one setting compared to the other ($P \leq 0.05$) are highlighted in bold. Adjusting for age and sex differences had no significant impact on these differences.

**P* values obtained from survey design-based logistic regression.

***P* values obtained from survey design-based linear regression.

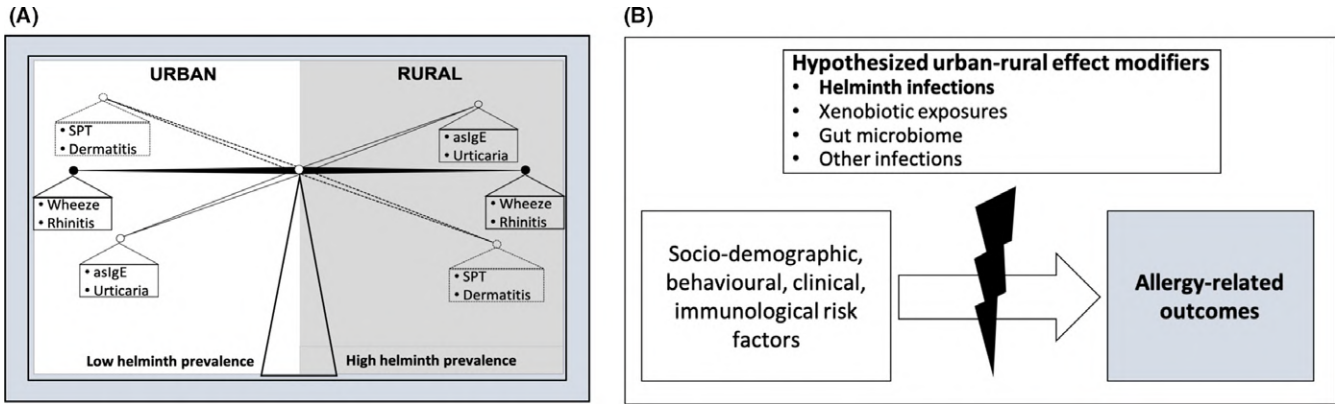

FIGURE 2 Urban-rural differences in risk factors for allergy-related outcomes in Uganda: a role for helminths? A, summary of principal findings regarding prevalence of allergy-related outcomes in urban Uganda and in rural Ugandan fishing communities. B, Risk factors for allergy-related outcomes differed between urban and rural settings. Our data suggest that helminth exposure is unlikely to be the only factor involved in this effect modification. Additional hypothesized effect modifiers are indicated

TABLE 2 Crude associations between allergy-related outcomes

		SPT	Wheeze	Rhinitis	Urticaria
asIgE					
Urban	OR (95% CI)	**21.4 (10.2, 44.6)**	5.5 (0.4, 68.6)	**3.7 (1.2, 11.9)**	3.7 (0.8, 16.2)
	P value	**<0.001**	0.171	**0.028**	0.075
	PAF (95% CI)	86.1% (81.4, 88.3)	65.5% (−120, 78.8)	53.1% (12.1, 66.6)	53.1% (−18.2, 68.2)
Rural	OR (95% CI)	**10.3 (5.3, 19.8)**	**3.9 (1.3, 11.5)**	1.1 (0.5, 2.6)	0.9 (0.6, 1.3)
	P value	**<0.001**	**0.015**	0.793	0.651
	PAF (95% CI)	80.9% (72.7, 85.1)	62.2% (19.3, 76.5)	5.7% (−57.9, 35.6)	−4.7% (−35.8, 12.4)
	Interaction *P* value	0.127	0.792	0.081	**0.056**
SPT					
Urban	OR (95% CI)		2.2 (0.6, 8.1)	**6.5 (3.4, 12.5)**	**2.2 (1.6, 2.8)**
	P value		0.211	**<0.001**	**<0.001**
	PAF (95% CI)		23.4% (−28.6, 37.6)	54.2% (45.2, 58.9)	20.8% (14.4, 24.6)
Rural	OR (95% CI)		**3.0 (1.8, 5.1)**	**2.6 (1.7, 3.9)**	1.2 (0.9, 1.6)
	P value		**<0.001**	**<0.001**	0.243
	PAF (95% CI)		29.2% (17.6, 31.9)	23.4% (15.5, 27.9)	3.6% (−2.4, 20.9)
	Interaction *P* value		0.647	**0.019**	**0.005**
Wheeze					
Urban	OR (95% CI)			**7.4 (1.7, 33.2)**	**4.9 (1.1, 21.7)**
	P value			**0.011**	**0.035**
Rural	OR (95% CI)			**11.9 (5.7, 24.9)**	1.4 (0.6, 3.3)
	P value			**<0.001**	0.403
	Interaction *P* value			0.557	0.127
Rhinitis					
Urban	OR (95% CI)				**9.6 (5.6, 16.4)**
	P value				**<0.001**
Rural	OR (95% CI)				0.7 (0.3, 1.6)
	P value				0.429
	Interaction *P* value				**<0.001**

asIgE: ImmunoCAP IgE sensitization to any of *D pteronyssinus*, *A hypogaea*, or *B germanica* on ImmunoCAP; SPT: skin prick test reactivity to any of Dermatophagoides mix, *B tropicalis* or *B germanica*.

Odds ratios (ORs), *P* values and population attributable fractions (PAFs) were obtained from survey design-adjusted analyses. Visible flexural dermatitis was not assessed because it was rare. Significant associations are highlighted in bold. Interaction *P* values are shown to denote whether tests for interaction showed statistical evidence for urban-rural differences in associations between allergy-related outcomes, or not.

older siblings, being born in a city (compared to town or village) and bathing in lakewater. The positive association between HIV and rhinitis was stronger in the urban compared to the rural survey (interaction $P = 0.028$). No other significant interactions were observed.

We did not find any evidence to suggest that current *Sm* infection influenced associations between non-helminth-related risk factors and clinical allergy-related outcomes, and interactions between the rural and urban survey (Table S4, C-E).

4 | DISCUSSION

We show risk factors for allergy-related outcomes in proximate Ugandan rural and urban settings. The rural setting was characterized by a significantly higher prevalence of *Sm* and nematode infections compared to the urban setting. The prevalence of SPT reactivity and visible flexural dermatitis was lower, and that of asIgE sensitization and urticaria higher, in the rural compared to urban setting. Risk factors for these outcomes differed by setting. We investigated the hypothesis that rural-urban differences in risk factors for allergy were attributable to differences in current *Sm* exposure. Despite observations that the rural environment (and higher intensity *Sm* infection within it) was associated with reduced odds of SPT reactivity, statistical analyses did not confirm a mediating role for current *Sm* infection in the rural-urban differences, implying that other exposures may play important roles. Similarly, rural-urban differences in associations with clinical allergy outcomes could not categorically be attributed to differences in current *Sm* infection between the two settings.

TABLE 3 Factors associated with SPT reactivity to any of *Dermatophagoides* mix, *B tropicalis* or *B germanica*

	Urban			Rural			
Factor	**N (%)[a]**	**aOR (95% CI)[bc]**	***P***	**N (%)[a]**	**aOR (95% CI)[bd]**	***P***	**Interaction *P***
Age		**1.02 (1.00, 1.03)**	**0.035**		**1.02 (1.00, 1.03)**	**0.015**	0.384
Sex							
Male	132 (26)	1		285 (18)	1		
Female	170 (21)	0.71 (0.49, 1.02)	0.061	291 (20)	1.09 (0.79, 1.52)	0.558	**0.015**
Older siblings (Yes/No)							
No	73 (22)	1		113 (24)	1		
Yes	194 (23)	1.58 (0.90, 2.76)	0.103	341 (22)	0.76 (0.56, 1.03)	0.076	0.133
Occupation							
Student or child (not at school)	111 (20)	1		136 (13)	1		
Unemployed or housewife	63 (24)	1.21 (0.70, 2.08)		61 (22)	0.79 (0.34, 1.85)		
Agricultural, fishing or lake related	11 (20)	0.74 (0.29, 1.87)		273 (22)	0.83 (0.39, 1.72)		
Professional or service providers	82 (28)	1.26 (0.77, 2.06)	0.709	103 (25)	0.93 (0.54, 1.62)	0.932	0.473
Maternal tribe							
Central Uganda	127 (25)	1		212 (20)	1		
Other, Ugandan	113 (21)	0.82 (0.52, 1.30)		272 (19)	0.86 (0.59, 1.27)		
Non-Ugandan, African	26 (25)	**1.77 (1.17, 2.70)**	**0.015**	86 (18)	0.76 (0.44, 1.32)	0.613	0.127
Maternal history of allergies							
No	192 (21)	1		433 (20)	1		
Yes	34 (31)	1.68 (0.89, 3.18)	0.107	71 (15)	0.90 (0.58, 1.41)	0.644	**0.013**
Location of birth							
City	16 (37)	1		12 (21)	1		
Town	34 (28)	0.56 (0.30, 1.02)		57 (24)	0.75 (0.37, 1.52)		
Village	60 (21)	**0.34 (0.18, 0.61)**	**0.004**	397 (21)	0.61 (0.29, 1.28)	0.419	**0.041**
BCG scar							
No	67 (19)	1		228 (19)	1		
Yes	234 (24)	**2.22 (1.24, 3.97)**	**0.010**	345 (19)	1.31 (0.96, 1.79)	0.083	0.601
Lake contact							
Never	72 (18)	1					
Rarely	140 (27)	0.92 (0.50, 1.67)		22 (33)	1		
Once a month	29 (24)	0.78 (0.39, 1.61)					
Once a week	26 (23)	1.04 (0.42, 2.57)	0.896	47 (24)	1.04 (0.64, 1.68)		
Daily/almost daily				385 (22)	0.89 (0.54, 1.48)	0.499	
Bathe in water from lake?							
No	249 (23)	1		25 (36)	1		
Yes	18 (18)	0.75 (0.27, 2.06)	0.558	429 (22)	**0.41 (0.24, 0.71)**	**0.002**	0.172
Hand washing after toilet							
No	19 (12)	1		151 (23)	1		
Yes	248 (25)	4.67 (0.88, 24.8)	0.068	303 (22)	0.78 (0.59, 1.02)	0.068	**0.001**
SWA-specific IgG4[e]		1.04 (0.86, 1.24)	0.691		**0.77 (0.63, 0.94)**	**0.013**	**0.011**
SEA-specific IgE[e]		1.32 (0.90, 1.91)	0.135		0.58 (0.29, 1.16)	0.119	0.109
Sm infection (KK)							
Uninfected	221 (22)	1		376 (21)	1		
Infected	20 (26)	1.47 (0.76, 2.83)	0.239	127 (16)	**0.68 (0.47, 0.97)**	**0.038**	0.332
Sm infection intensity (KK)							
Uninfected	221 (22)	1		376 (21)	1		

(Continues)

TABLE 3 (Continued)

Factor	Urban			Rural			Interaction *P*
	N (%)[a]	aOR (95% CI)[bc]	*P*	N (%)[a]	aOR (95% CI)[bd]	*P*	
Light	15 (38)	**2.39 (1.24, 4.64)**		65 (16)	0.66 (0.43, 1.01)		
Moderate	3 (12)	0.76 (0.22, 2.61)		40 (18)	0.83 (0.52, 1.34)		
Heavy	2 (14)	0.55 (0.05, 6.81)	0.055	22 (12)	0.49 (0.22, 1.14)	0.053	**0.015**
Sm infection (PCR)							
Uninfected	188 (21)	1		289 (22)	1		
Infected	48 (25)	**1.57 (1.01, 2.43)**	**0.044**	214 (17)	**0.66 (0.49, 0.89)**	**0.010**	**0.002**
Sm infection (CCA)							
Negative	163 (24)	1		114 (27)	1		
Positive	115 (22)	1.19 (0.69, 2.06)	0.517	414 (18)	**0.56 (0.37, 0.83)**	**0.006**	0.184
Malaria treatment, last 12 mo							
No	163 (24)	1		234 (21)	1		
Yes	100 (22)	0.86 (0.52, 1.42)	0.536	323 (18)	1.08 (0.85, 1.38)	0.502	0.730
HIV							
Negative	272 (22)	1		380 (19)	1		
Positive	19 (32)	1.82 (0.56, 5.93)	0.302	98 (25)	1.17 (0.74, 1.85)	0.495	0.440

Associations shown in this table are from adjusted analyses. Full table with crude associations is shown in supplementary Table S1. This table shows only factors that were associated with SPT reactivity (before and/or after adjustment) in either the urban or the rural survey. All other factors that were assessed are listed in the statistical methods section. Significant associations are highlighted in bold. Interaction *P* values are shown to establish whether associations between potential risk factors and SPT reactivity differed, or not, given the setting.

aOR: adjusted odds ratios; CCA: circulating cathodic antigen; KK: Kato-Katz; PCR: Polymerase Chain Reaction; SEA: Schistosoma egg antigen; SWA: Schistosoma adult worm antigen.

[a]Number (percentage in parenthesis) of SPT reactive individuals in each category.

[b]Odds ratios (ORs) and 95% confidence intervals (CI) were adjusted for survey design.

[c]ORs were adjusted for location of birth, BCG scar, hand washing after toilet use, alcohol use, age and sex.

[d]ORs were adjusted for HIV infection status, maternal history of allergies, recent malaria treatment, presence/absence of older siblings, age and sex.

[e]Log10 (concentration+1) transformation applied before analysis.

Our rural and urban settings were atypical. Observations in the rural survey are against a backdrop of three years of well-organized community-level anthelminthic intervention[25] that led to a decline in helminth intensity in both standard and intensive treatment arms, but had no effect on overall *Sm* prevalence.[26] Before analysis of risk factors, we confirmed a lack of effect of the intensive (compared with standard) anthelminthic treatment on allergy-related outcomes. The urban survey was done in the unusual context of a setting with considerable exposure to light *Sm* infection (inferred from 44% urine CCA positivity). However, this enabled us to adjust for *Sm* infection in both settings and hence explore the role of *Sm* in interactions between the settings. Recruitment of participants in the urban survey was done after conclusion of the rural survey; however, this is unlikely to account for observed urban-rural differences in allergy risk factors, as both surveys were conducted by the same research team, and covered approximately 1 year (so any seasonal effects were approximately matched). Another potential limitation was the large number of statistical tests, increasing likelihood of chance findings. However, we were cautious to look for patterns of association rather than interpreting individual results equally.

In keeping with the "old friends" hypothesis[1] and observations from several studies,[37-39] SPT reactivity was less prevalent in the helminth-endemic rural setting and was inversely associated with helminth infections in the same setting. The only exception was *Trichuris trichiura* infection, which was weakly positively associated with *Dermatophagoides* SPT (Table S3). This lone observation was also manifest in the same communities in a baseline household survey 3 years earlier,[24] although no other helminth species were associated with SPT then. The current observations beg further investigation into the impact of anthelminthic treatment on SPT-helminth associations in a helminth-endemic setting. In mice, allergic airway inflammation is increased during acute *Sm* infection but reduces drastically with progression to chronic infection.[40] In our urban setting, light *Sm* infection was positively associated with SPT reactivity while moderate and heavy infections were inversely associated with the same outcome (Table 3). "Helminth-related" behavioural characteristics were also inversely associated with SPT reactivity in the rural survey. It is plausible that in these fishing communities, frequent lake contact, bathing in lakewater and handwashing, for example, increase the risk for *Sm* infection through contact with infected snails. Indeed, these characteristics were strongly associated with *Sm* infection ($P < 0.001$). However, the same characteristics were also inversely associated with asIgE sensitization in the rural survey but not in the urban survey.

As discussed earlier, Pinot de Moira and colleagues' study in a Ugandan village found that hookworm infection abrogated the

TABLE 4 Factors associated with IgE sensitization (ImmunoCAP IgE > 0.35 kU/L) to any of *D pteronyssinus*, *A hypogaea* or *B germanica*

	Urban			Rural			
Factor	**N (%)[a]**	**aOR (95% CI)[bd]**	***P***	**N (%)[a]**	**aOR (95% CI)[bc]**	***P***	**Interaction *P***
Age		0.99 (0.98, 1.01)	0.547		1.01 (0.98, 1.03)	0.589	0.728
Sex							
Male	47 (48)	1		241 (64)	1		
Female	101 (41)	0.77 (0.51, 1.15)	0.200	196 (49)	0.69 (0.42, 1.14)	0.140	0.407
Younger siblings (Yes/No)							
No	27 (33)	1		61 (62)	1		
Yes	106 (46)	**2.07 (1.07, 4.01)**	**0.030**	313 (56)	0.76 (0.53, 1.09)	0.129	**0.008**
Occupation							
Student or child (not at school)	68 (48)	1		64 (51)	1		
Unemployed or housewife	34 (40)	0.70 (0.31, 1.60)		58 (54)	**2.05 (1.38, 3.03)**		
Agricultural, fishing or lake related	4 (36)	0.56 (0.13, 2.46)		251 (61)	**1.87 (1.04, 3.37)**		
Professional or service providers	27 (38)	0.61 (0.26, 1.43)	0.725	61 (47)	1.38 (0.72, 2.66)	**0.014**	0.148
Lake contact							
Never	39 (45)	1					
Rarely	69 (42)	0.98 (0.52, 1.85)		8 (42)	1		
Once a month	11 (35)	0.74 (0.38, 1.41)					
Once a week	14 (50)	1.32 (0.49, 3.55)	0.835	25 (44)	0.82 (0.23, 2.89)		
Daily/almost daily				343 (59)	1.64 (0.45, 5.90)	0.174	
Bathe in water from lake?							
No	121 (42)	1		15 (63)	1		
Yes	12 (57)	1.86 (0.50, 6.87)	0.331	361 (57)	0.42 (0.15, 1.11)	0.078	0.065
Hand washing after toilet							
No	15 (39)	1		148 (71)	1		
Yes	117 (43)	1.36 (0.69, 2.66)	0.344	228 (50)	**0.43 (0.30, 0.61)**	**<0.001**	**0.003**
SWA-specific IgE[e]		2.95 (0.51, 17.2)	0.214		**6.17 (2.79, 13.6)**	**<0.001**	0.459
SWA-specific IgG4[e]		1.01 (0.85, 1.19)	0.932		1.07 (0.89, 1.27)	0.470	0.433
SEA-specific IgG4[e]		1.11 (0.97, 1.25)	0.102		1.07 (0.95, 1.21)	0.227	0.704
SWA-specific IgG[e]		**3.33 (1.12, 9.86)**	**0.031**		1.53 (0.82, 2.86)	0.177	0.340
SEA-specific IgG[e]		1.77 (0.63, 4.96)	0.260		1.43 (0.88, 2.30)	0.138	0.796
S mansoni infection (KK)							
Uninfected	119 (44)	1		271 (55)	1		
Infected	6 (43)	1.06 (0.34, 3.35)	0.910	118 (63)	**1.52 (1.19, 1.94)**	**0.002**	0.180
S mansoni infection intensity (KK)							
Uninfected	119 (44)	1		271 (55)	1		
Light	2 (29)	0.66 (0.11, 4.04)		54 (57)	**1.74 (1.18, 2.54)**		
Moderate	2 (50)	1.20 (0.16, 8.79)		35 (66)	0.94 (0.46, 1.90)		
Heavy	2 (67)	2.32 (0.13, 40.9)	0.662	29 (73)	2.37 (0.71, 7.83)	**0.028**	0.536
Any nematode infection[f]							
No	109 (42)	1		281 (54)	1		
Yes	16 (62)	2.34 (0.76, 7.19)	0.130	108 (66)	1.53 (0.94, 2.49)	0.084	0.287

(Continues)

predicted association between *Dermatophagoides*-specific IgE and basophil histamine release.[22] We postulated that the rural setting might interfere with the link between atopic sensitization (asIgE, SPT) and clinical outcomes (reported wheeze and rhinitis) through high helminth exposure. Indeed, we found that associations between asIgE or SPT sensitization and clinical outcomes were weak among participants from the rural compared to the urban setting. However, statistical analyses did not suggest that this

TABLE 4 (Continued)

Factor	Urban			Rural			Interaction *P*
	N (%)[a]	aOR (95% CI)[bd]	*P*	N (%)[a]	aOR (95% CI)[bc]	*P*	
Slept under mosquito net last night?							
No	35 (45)	1		203 (62)	1		
Yes	97 (42)	0.93 (0.52, 1.66)	0.958	172 (52)	**0.63 (0.41, 0.97)**	**0.037**	0.316
Malaria treatment, last 12 mo							
No	83 (45)	1		202 (63)	1		
Yes	48 (39)	0.78 (0.46, 1.35)	0.365	221 (51)	**0.52 (0.34, 0.81)**	**0.005**	0.185

Associations shown in this table are from adjusted analyses. Full table with crude associations is shown in supplementary Table S1. This table shows only factors that were associated with IgE sensitization (before and/or after adjustment) in either the urban or the rural survey. All other factors that were assessed are listed in the statistical methods section. Significant associations are highlighted in bold. Interaction *P* values are shown to denote whether tests for interaction showed statistical evidence for urban-rural differences in associations with IgE sensitization, or not.

aOR: adjusted odds ratios; KK: Kato-Katz; SWA: Schistosoma adult worm antigen; SEA: Schistosoma egg antigen.

[a]Number (percentage in parenthesis) of IgE sensitized individuals in each category.

[b]Odds ratios (ORs) and 95% confidence intervals (CI) adjusted for survey design.

[c]All ORs were adjusted for hand washing after toilet use, mosquito net use, malaria treatment, age and sex.

[d]All ORs were adjusted for age and sex.

[e]Log10 (concentration+1) transformation applied before analysis.

[f]Infection with any of *Ascaris lumbricoides*, *Trichuris trichiura (assessed by KK)*, *Necator americanus*, *Strongyloides stercoralis* (assessed by PCR).

difference was mediated by current *Sm* infection. Furthermore, the PAF for SPT associated with asIgE in both the helminth-endemic rural survey and in the urban survey was high, and adjusting for *Sm* or *Schistosoma*-specific antibodies had no effect on this association.

Allergen-specific IgE sensitization, particularly to cockroach, was more prevalent in the rural compared to the urban setting, possibly due to the higher helminth prevalence in the former. Additionally, helminth infections and *Schistosoma*-specific antibody levels were positively associated with asIgE in both surveys. Our immunoassays measured IgE sensitization to crude allergen extracts; these may contain cross-reactive components that are conserved in several helminth antigens,[41-46] explaining the above associations. These cross-reactive components may be less effective at mediating the effector phase of the allergic response, explaining the lower prevalence of SPT reactivity in the helminth-endemic rural survey.

Associations with wheeze and rhinitis should be interpreted with caution, because these outcomes were relatively rare. Furthermore, reported wheeze can easily be misclassified in these populations, because there is no direct translation of the word "wheeze" in the local languages.[24,47] Nonetheless, rural-urban differences in the risk factors for these outcomes were visible. For example, while *Schistosoma*-specific antibody levels were inversely associated with wheezing in the urban survey, the reverse was true in the rural setting. Urticarial rash was a more common outcome, particularly in the helminth-endemic rural survey, where it may be indicative of parasite-induced skin allergy[48] and reaction to parasite antigens following anthelminthic treatment.[49] Support for these deductions comes from our observations that recent anthelminthic treatment (urban survey) and SEA-specific IgE (rural survey) were associated with urticaria.

In conclusion, we show that risk factors for allergy-related outcomes differ between rural and urban communities in this tropical setting. However, our analyses did not confirm a role for current helminth (*Sm*) infection as the primary mechanism of the observed effect modification between the two settings, despite indicative trends. Differences in other environmental exposures may contribute significantly.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AME conceived the LaVIISWA study and the urban survey. GN, AME, MY and RvR designed the laboratory studies. GN, JK, JN and SV performed the laboratory experiments. AME, RES, MN, PNK, JT, CZ, RK, EN, HM and CN led and participated in field and clinic procedures. GN analysed the results with significant input from LL, ELW, HM, MY and AME. GN wrote the manuscript, with all authors contributing to the interpretation of the results, and revision and approval of the final manuscript. GN is the guarantor of the article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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4.3 Supplementary information for Research paper 1 (also available in the article's online repository at https://onlinelibrary.wiley.com/journal/13652222)

4.3.1 Supplementary methods

4.3.1.1 S. mansoni adult worm (SWA)- and egg (SEA)-specific IgE and IgG4 ELISA

All but the first 2 columns of 4HBX Immulon (Thermo Scientific, NY, USA) 96-well plates were coated with 50µl of SWA [8 µg/ml] or SEA [2.4 µg/ml] (purchased from Professor Michael J Doenhoff, University of Nottingham) in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer (0.1M, pH 9.6). Two-fold dilutions of human IgE (Calbiochem, Beeston, UK) or IgG4 (Sigma-Aldrich) standard, diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4^0C. Plates were washed with phosphate-buffered saline (PBS 1X)-tween 20 solution, blocked with 150µl of 1% skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4^0C with 50µl of plasma samples diluted 1/20 (IgE assay) or 1/200 (IgG4 assay) with 0.1% skimmed milk in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates overnight at 4^0C with 0.5µg/ml of biotinylated monoclonal mouse anti-human IgE or IgG4 (BD Pharmingen™). This was followed by a 1-hour incubation with a streptavidin-Horseradish Peroxidase (strep-HRP) conjugate (Mast Group Ltd, Bootle, UK), diluted 1/4000 with assay buffer, at RT. Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped after 30 minutes with 25µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgE or IgG4 concentrations (ng/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

4.3.1.2 S. mansoni adult worm (SWA)- and egg (SEA)-specific IgG ELISA

All but the first 2 columns of 4HX Immulon (VWR, UK, Cat No 735-0465) 96-well plates were coated with 50µl of SWA [8 µg/ml] or SEA [2.4 µg/ml] (purchased from Professor Michael J

Doenhoff, University of Nottingham) in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer (0.1M, pH 9.6). Two-fold dilutions of human IgG (Sigma-Aldrich), diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4^0C. Plates were washed with phosphate-buffered saline (PBS)-tween 20 solution, blocked with 150µl of 1% skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4^0C with 50µl of plasma samples diluted 1/3000 with 0.1% skimmed milk in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates for 1 hour at RT with 0.5µg/ml of polyclonal rabbit anti- human IgG/HRP (Dako, Denmark). Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped after 30 minutes with 25µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgG concentrations (ng/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

4.3.2 Supplementary tables

Table S1. Crude versus *Sm*-adjusted associations between allergy-related outcomes

			SPT		WHEEZE		RHINITIS		URTICARIA	
			Unadjusted	Adjusted for *Sm* (CCA)*	Unadjusted	Adjusted for *Sm* (CCA)*	Unadjusted	Adjusted for *Sm* (CCA)*	Unadjusted	Adjusted for *Sm* (CCA)*
asIgE	**URBAN**	OR (95% CI) p value	**21.4 (10.2, 44.6)** **<0.001**	**23.6 (10.2, 54.4)** **<0.001**	5.5 (0.4, 68.6) 0.171	4.4 (0.4, 47.3) 0.210	**3.7 (1.2, 11.9)** **0.028**	**3.7 (1.1, 12.1)** **0.035**	3.7 (0.8, 16.2) 0.075	3.4 (0.8, 14.5) 0.092
	RURAL	OR (95% CI) p value	**10.3 (5.3, 19.8)** **<0.001**	**10.1 (4.9, 20.9)** **<0.001**	**3.9 (1.3, 11.5)** **0.015**	3.0 (0.9, 9.5) 0.061	1.1 (0.5, 2.6) 0.793	0.99 (0.3, 2.8) 0.979	0.9 (0.6, 1.3) 0.651	0.9 (0.6, 1.3) 0.659
		Interaction p	0.127	0.074	0.792	0.817	0.081	0.073	0.056	0.089
SPT	**URBAN**	OR (95% CI) p value			2.2 (0.6, 8.1) 0.211	1.7 (0.5, 6.1) 0.366	**6.5 (3.4, 12.5)** **<0.001**	**5.9 (3.1, 11.4)** **<0.001**	**2.2 (1.6, 2.8)** **<0.001**	**2.3 (1.7, 3.0)** **<0.001**
	RURAL	OR (95% CI) p value			**3.0 (1.8, 5.1)** **<0.001**	**3.6 (1.9, 6.8)** **<0.001**	**2.6 (1.7, 3.9)** **<0.001**	**2.7 (1.7, 4.1)** **<0.001**	1.2 (0.9, 1.6) 0.243	1.03 (0.7, 1.4) 0.845
		Interaction p			0.647	0.281	**0.019**	**0.037**	**0.005**	**0.001**
WHEEZE	**URBAN**	OR (95% CI) p value					**7.4 (1.7, 33.2)** **0.011**	**7.1 (1.5, 32.8)** **0.015**	**4.9 (1.1, 21.7)** **0.035**	**6.1 (1.4, 27.3)** **0.020**
	RURAL	OR (95% CI) p value					**11.9 (5.7, 24.9)** **<0.001**	**14.9 (6.8, 32.5)** **<0.001**	1.4 (0.6, 3.3) 0.403	1.2 (0.6, 2.7) 0.588
		Interaction p					0.557	0.375	0.127	0.056
RHINITIS	**URBAN**	OR (95% CI) p value							**9.6 (5.6, 16.4)** **<0.001**	**10.4 (5.4, 19.7)** **<0.001**
	RURAL	OR (95% CI) p value							0.7 (0.3, 1.6) 0.429	0.6 (0.2, 1.7) 0.321
		Interaction p							**<0.001**	**<0.001**

Odds ratios (ORs) and p values were obtained from survey design-adjusted analyses. Visible flexural dermatitis was not assessed because it was rare. Significant associations are highlighted in bold.

*Adjusting for other *Sm* infection variables (KK / PCR), *Sm* infection intensity (KK) and SWA- and SEA-specific antibodies paints a similar picture.

asIgE: ImmunoCAP IgE sensitisation to any of *D. pteronyssinus, A. hypogaea,* or *B. germanica* on ImmunoCAP; **SPT**: skin prick test reactivity to any of *Dermatophagoides* mix, *B. tropicalis* or *B. germanica*; **CCA:** Circulating Cathodic Antigen

Table S2. Crude and adjusted associations with SPT reactivity to any of *Dermatophagoides* mix, *B. tropicalis* or *B. germanica*

	UNADJUSTED ANALYSIS						ADJUSTED ANALYSIS				
	URBAN			**RURAL**			**URBAN**		**RURAL**		
Factor	N (%)[≠]	OR (95% CI)¶	p	N (%)[≠]	OR (95% CI)¶	p	OR (95% CI) ¶#	p	OR (95% CI)¶§	p	Interaction p
Age		**1.01 (1.00, 1.02)**	**0.005**		**1.02 (1.01, 1.03)**	**<0.001**	**1.02 (1.00, 1.03)**	**0.035**	**1.02 (1.00, 1.03)**	**0.015**	0.384
Sex											
Male	132 (26)	1		285 (18)	1		1		1		
Female	170 (21)	0.77 (0.59, 1.02)	0.068	291 (20)	1.00 (0.74, 1.37)	0.979	0.71 (0.49, 1.02)	0.061	1.09 (0.79, 1.52)	0.558	**0.015**
Older siblings (Yes/No)											
No	73 (22)	1		113 (24)	1		1		1		
Yes	194 (23)	1.05 (0.78, 1.42)	0.691	341 (22)	**0.75 (0.58, 0.98)**	**0.036**	1.58 (0.90, 2.76)	0.103	0.76 (0.56, 1.03)	0.076	0.133
Occupation											
Student or child (not at school)	111 (20)	1		136 (13)	1		1		1		
Unemployed or housewife	63 (24)	1.22 (0.92, 1.63)		61 (22)	**1.68 (1.09, 2.58)**		1.21 (0.70, 2.08)		0.79 (0.34, 1.85)		
Agricultural, fishing or lake related	11 (20)	0.98 (0.44, 2.21)		273 (22)	**1.78 (1.41, 2.25)**		0.74 (0.29, 1.87)		0.83 (0.39, 1.72)		
Professional or service providers	82 (28)	1.57 (1.16, 2.14)	0.059	103 (25)	**1.96 (1.25, 3.06)**	**<0.001**	1.26 (0.77, 2.06)	0.709	0.93 (0.54, 1.62)	0.932	0.473
Maternal tribe											
Central Uganda	127 (25)	1		212 (20)	1		1		1		
Other, Ugandan	113 (21)	0.78 (0.59, 1.03)		272 (19)	1.03 (0.76, 1.35)		0.82 (0.52, 1.30)		0.86 (0.59, 1.27)		
Non-Ugandan, African	26 (25)	1.01 (0.59, 1.71)	0.179	86 (18)	1.03 (0.79, 1.32)	0.977	**1.77 (1.17, 2.70)**	**0.015**	0.76 (0.44, 1.32)	0.613	0.127
Maternal history of allergies											
No	192 (21)	1		433 (20)	1		1		1		
Yes	34 (31)	1.65 (0.95, 2.86)	0.073	71 (15)	**0.75 (0.58, 0.95)**	**0.018**	1.68 (0.89, 3.18)	0.107	0.90 (0.58, 1.41)	0.644	**0.013**
Location of birth											
City	16 (37)	1		12 (21)	1		1		1		
Town	34 (28)	0.64 (0.38, 1.09)		57 (24)	0.90 (0.42, 1.95)		0.56 (0.30, 1.02)		0.75 (0.37, 1.52)		
Village	60 (21)	**0.44 (0.28, 0.68**)	**0.005**	397 (21)	0.89 (0.49, 1.61)	0.932	**0.34 (0.18, 0.61)**	**0.004**	0.61 (0.29, 1.28)	0.419	**0.041**

BCG scar											
No	67 (19)	1		228 (19)	1		1		1		
Yes	234 (24)	**1.39 (1.05, 1.83)**	**0.021**	345 (19)	1.07 (0.89, 1.28)	0.444	**2.22 (1.24, 3.97)**	**0.010**	1.31 (0.96, 1.79)	0.083	0.601
Lake contact											
Never	72 (18)	1					1				
Rarely	140 (27)	**1.64 (1.36, 1.99)**		22 (33)	1		0.92 (0.50, 1.67)		1		
Once a month	29 (24)	1.42 (0.99, 2.02)					0.78 (0.39, 1.61)				
Once a week	26 (23)	1.37 (0.88, 2.13)	**0.021**	47 (24)	0.73 (0.44, 1.22)		1.04 (0.42, 2.57)	0.896	1.04 (0.64, 1.68)		
Daily/ almost daily				385 (22)	**0.65 (0.44, 0.96)**	**0.033**			0.89 (0.54, 1.48)	0.499	
Bathe in water from lake?											
No	249 (23)	1		25 (36)	1		1		1		
Yes	18 (18)	0.74 (0.32, 1.67)	0.447	429 (22)	**0.38 (0.18, 0.80)**	**0.013**	0.75 (0.27, 2.06)	0.558	**0.41 (0.24, 0.71)**	**0.002**	0.172
Hand washing after toilet											
No	19 (12)	1		151 (23)	1		1		1		
Yes	248 (25)	**2.49 (1.77, 3.49)**	**<0.001**	303 (22)	0.88 (0.68, 1.13)	0.309	4.67 (0.88, 24.8)	0.068	0.78 (0.59, 1.02)	0.068	**0.001**
SWA-specific IgG4*		1.03 (0.94, 1.13)	0.449		0.87 (0.75, 1.02)	0.093	1.04 (0.86, 1.24)	0.691	**0.77 (0.63, 0.94)**	**0.013**	**0.011**
SEA-specific IgE*		**1.47 (1.02, 2.11)**	**0.038**		1.03 (0.58, 1.81)	0.923	1.32 (0.90, 1.91)	0.135	0.58 (0.29, 1.16)	0.119	0.109
Sm infection (KK)											
Uninfected	221 (22)	1		376 (21)	1		1		1		
Infected	20 (26)	1.22 (0.62, 2.41)	0.552	127 (16)	**0.69 (0.53, 0.91)**	**0.010**	1.47 (0.76, 2.83)	0.239	**0.68 (0.47, 0.97)**	**0.038**	0.332
Sm infection intensity (KK)											
Uninfected	221 (22)	1		376 (21)	1		1		1		
Light	15 (38)	**2.2 (1.09, 4.46)**		65 (16)	**0.71 (0.53, 0.95)**		**2.39 (1.24, 4.64)**		0.66 (0.43, 1.01)		
Moderate	3 (12)	0.48 (0.07, 3.03)		40 (18)	0.76 (0.49, 1.18)		0.76 (0.22, 2.61)		0.83 (0.52, 1.34)		
Heavy	2 (14)	0.58 (0.13, 2.75)	0.772	22 (12)	**0.55 (0.34, 0.91)**	**0.017**	0.55 (0.05, 6.81)	0.055	0.49 (0.22, 1.14)	0.053	**0.015**
Sm infection (PCR)											
Uninfected	188 (21)	1		289 (22)	1		1		1		
Infected	48 (25)	1.26 (0.94, 1.70)	0.117	214 (17)	**0.73 (0.55, 0.96)**	**0.031**	**1.57 (1.01, 2.43)**	**0.044**	**0.66 (0.49, 0.89)**	**0.010**	**0.002**
Sm infection (CCA)											
Negative	163 (24)	1		114 (27)	1		1		1		

	Positive	115 (22)	0.89 (0.64, 1.26)	0.508	414 (18)	**0.59 (0.44, 0.80)**	**0.001**	1.19 (0.69, 2.06)	0.517	**0.56 (0.37, 0.83)**	**0.006**	0.184
Malaria treatment, last 12months												
	No	163 (24)	1		234 (21)	1		1		1		
	Yes	100 (22)	0.91 (0.72, 1.16)	0.461	323 (18)	**0.82 (0.68, 0.97)**	**0.027**	0.86 (0.52, 1.42)	0.536	1.08 (0.85, 1.38)	0.502	0.730
HIV												
	Negative	272 (22)	1		380 (19)	1		1		1		
	Positive	19 (32)	1.61 (0.97, 2.64)	0.060	98 (25)	**1.47 (1.04, 2.08)**	**0.031**	1.82 (0.56, 5.93)	0.302	1.17 (0.74, 1.85)	0.495	0.440

Table shows only factors that were associated with SPT reactivity (before and/or after adjustment) in either the urban or the rural survey. All other factors that were assessed are listed in the statistical methods section. Significant associations are highlighted in bold. Interaction p values are shown to denote whether tests for interaction, using the adjusted model, showed statistical evidence for urban-rural differences in associations with SPT reactivity, or not.

≠Number (percentage in parenthesis) of SPT reactive individuals in each category

¶Odds ratios (ORs) and 95% confidence intervals (CI) were adjusted for survey design

#ORs were adjusted for location of birth, BCG scar, hand washing after toilet use, alcohol use, age and sex.

§ORs were adjusted for HIV infection status, maternal history of allergies, recent malaria treatment, presence/absence of older siblings, age and sex.

*Log_{10} (concentration+1) transformation applied before analysis.

KK: Kato-Katz; PCR: Polymerase Chain Reaction; CCA: Circulating Cathodic Antigen; SWA: Schistosoma adult worm antigen; SEA: Schistosoma egg antigen.

Table S3. Summary of risk factors for SPT reactivity to individual allergen extracts

	URBAN SURVEY		RURAL SURVEY	
Outcome	**Factors positively associated with outcome**	**Factors inversely associated with outcome**	**Factors positively associated with outcome**	**Factors inversely associated with outcome**
SPT reactivity, *Dermatophagoides* mix	• *S. mansoni* infection [PCR] (p=0.062) and intensity (p for trend=0.007) • Presence of older siblings (p=0.005)	• Female gender (p=0.013) • Recent malaria treatment (p<0.001)	• *T. trichiura* infection (p=0.046) • Age (p=0.015) • Type of footwear (sandals or shoes, versus bare feet) [p=0.034]	• *S. mansoni* infection [PCR] (p<0.001) • *S. mansoni* infection [KK] (p=0.001) • *S. mansoni* infection intensity [KK] (p<0.001) • SWA-specific IgG4 (p=0.001) • Bathing in lake water (p=0.016) • SEA-specific IgG (p=0.046)
SPT reactivity, *B. tropicalis*	• Age (p=0.032) • Maternal history of allergies (p=0.004) • Hand washing after toilet use (p=0.013) • SEA-specific IgE (p=0.012)	• Paternal tribe (p=0.001)	-	• *S. mansoni* infection [KK] (p=0.016) • *S. mansoni* infection intensity [KK] (p=0.006) • SEA-specific IgG (p=0.012) • SWA-specific IgG (p=0.041) • SEA-specific IgG4 (p=0.009) • Bathing in lake water (p<0.001) • Presence of older siblings (p=0.034) • Daily contact with lake (versus weekly or rarely) [p for trend =0.026]

SPT reactivity, *B. germanica*	• BCG scar (p=0.017) • Maternal history of allergies (p=0.019) • Presence of older siblings (p=0.033) • SWA-specific IgG4 (p=0.046)	Location of birth (p for trend=0.049)	• Age (p<0.001)	• Presence of older siblings (p=0.043)

P values are from adjusted analyses, conducted as described in the main text. The following were assessed as potential risk factors: age, sex, presence of older/younger siblings, maternal tribe, paternal tribe, location of birth, occupation, frequency of lake contact, type of bathing water, hand-washing behaviour [before eating, after toilet use], footwear outside the house, smoking, alcohol use, helminth infections, exposure to anthelminthic treatment in utero, anthelminthic treatment in last 12 months, maternal and paternal history of allergy/eczema/asthma, BCG scar, immunization history, treatment for malaria in last 12 months, malaria infection, HIV infection and plasma concentration of SEA- and SWA-specific IgE, IgG4 and IgG antibodies.

Table S4. Effect of adjusting for *Sm* infection on associations between non-helminth-related factors and allergy-related outcomes

A. SPT reactivity to *Dermatophagoides mix, B. tropicalis or B. germanica*

Factor	URBAN		RURAL			
	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	Interaction p	Interaction p**
Age	**1.02 (1.00, 1.03)**	**1.02 (1.00, 1.03)**	**1.02 (1.00, 1.03)**	1.01 (0.99, 1.03)	0.384	0.544
Sex						
Male	1	1	1	1		
Female	0.71 (0.49, 1.02)	0.74 (0.52, 1.05)	1.09 (0.79, 1.52)	0.95 (0.67, 1.35)	**0.015**	0.057
Older siblings (Yes/No)						
No	1	1	1	1		
Yes	1.58 (0.90, 2.76)	1.46 (0.83, 2.58)	0.76 (0.56, 1.03)	**0.69 (0.51, 0.96)**	0.133	0.139
Maternal tribe						
Central Uganda	1	1	1	1		
Other, Ugandan	0.82 (0.52, 1.30)	0.79 (0.49, 1.30)	0.86 (0.59, 1.27)	0.81 (0.53, 1.22)		
Non-Ugandan	**1.77 (1.17, 2.70)**	**1.83 (1.23, 2.71)**	0.76 (0.44, 1.32)	0.75 (0.41, 1.36)	0.127	0.088
Maternal history of allergies						
No	1	1	1	1		
Yes	1.68 (0.89, 3.18)	**2.2 (1.02, 4.96)**	0.90 (0.58, 1.41)	0.95 (0.59, 1.51)	**0.013**	**0.013**
Location of birth						
City	1	1	1	1		
Town	0.56 (0.30, 1.02)	0.70 (0.35, 1.38)	0.75 (0.37, 1.52)	0.82 (0.36, 1.85)		
Village	**0.34 (0.18, 0.61)**	**0.39 (0.23, 0.67)**	0.61 (0.29, 1.28)	0.62 (0.28, 1.35)	**0.041**	0.086
BCG scar						
No	1	1	1	1		
Yes	**2.22 (1.24, 3.97)**	**2.12 (1.17, 3.84)**	1.31 (0.96, 1.79)	1.35 (0.95, 1.91)	0.601	0.863
Handwashing after toilet use						
No	1	1	1	1		

Yes	4.67 (0.88, 24.8)	3.83 (0.73, 20.2)	0.78 (0.59, 1.02)	**0.76 (0.59, 0.96)**	**0.001**	**0.005**
Malaria treatment, last 12months						
No	1	1	1	1		
Yes	0.86 (0.52, 1.42)	0.91 (0.52, 1.60)	1.08 (0.85, 1.38)	1.11 (0.87, 1.41)	0.730	0.885
HIV						
Negative	1	1	1	1		
Positive	1.82 (0.56, 5.93)	1.86 (0.58, 5.90)	1.17 (0.74, 1.85)	1.12 (0.71, 1.79)	0.440	0.693
B. IgE sensitisation (ImmunoCAP IgE > 0.35 kU/L) to any of *D. pteronyssinus*, *A. hypogaea* or *B. germanica*						
Factor	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	Interaction p	Interaction p**
Age	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	1.01 (0.98, 1.03)	1.01 (0.98, 1.03)	0.728	0.999
Sex						
Male	1	1	1	1		
Female	0.77 (0.51, 1.15)	0.73 (0.52, 1.03)	0.69 (0.42, 1.14)	0.74 (0.43, 1.29)	0.407	0.511
Younger siblings (Yes/No)						
No	1	1	1	1		
Yes	**2.07 (1.07, 4.01)**	**2.23 (1.17, 4.23)**	0.76 (0.53, 1.09)	0.72 (0.46, 1.12)	**0.008**	**0.002**
Handwashing after toilet use						
No	1	1	1	1		
Yes	1.36 (0.69, 2.66)	1.42 (0.67, 2.96)	**0.43 (0.30, 0.61)**	**0.41 (0.28, 0.61)**	**0.003**	**0.003**
Slept under mosquito net last night?						
No	1	1	1	1		
Yes	0.93 (0.52, 1.66)	0.89 (0.48, 1.66)	**0.63 (0.41, 0.97)**	**0.61 (0.39, 0.95)**	0.316	0.317
Malaria treatment, last 12months						
No	1	1	1	1		
Yes	0.78 (0.46, 1.35)	0.79 (0.43, 1.45)	**0.52 (0.34, 0.81)**	**0.55 (0.34, 0.87)**	0.185	0.300

C. Self-reported recent wheeze

Factor	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	Interaction p	Interaction p**
Age	1.01 (0.99, 1.02)	1.02 (1.00, 1.03)	1.00 (1.00, 1.03)	1.03 (1.01, 1.04)	0.386	0.378
Sex						
Male	1	1	1	1		
Female	1.30 (0.55, 3.07)	1.55 (0.82, 2.90)	**0.47 (0.31, 0.73)**	**0.55 (0.34, 0.89)**	0.087	0.057
Older siblings (Yes/No)						
No	1	1	1	1		
Yes	**0.36 (0.19, 0.73)**	**0.29 (0.11, 0.77)**	0.88 (0.43, 1.79)	0.91 (0.31, 2.67)	0.177	0.108
Maternal history of allergies						
No	1	1	1	1		
Yes	3.34 (0.82, 13.6)	2.62 (0.77, 8.92)	1.11 (0.45, 2.71)	1.25 (0.47, 3.35)	0.083	0.162
Paternal history of allergies						
No	1	1	1	1		
Yes	4.96 (0.81, 30.4)	5.30 (0.77, 36.1)	2.32 (0.97, 5.49)	**3.29 (1.34, 8.10)**	0.259	0.437
Paternal tribe						
Central Uganda	1	1	1	1		
Other, Ugandan	1.58 (0.35, 7.16)	1.22 (0.27, 5.48)	0.66 (0.38, 1.18)	0.62 (0.27, 1.41)		
Non-Ugandan	**4.25 (1.94, 9.34)**	**5.59 (2.19, 14.2)**	0.61 (0.36, 1.15)	0.46 (0.19, 1.06)	**<0.001**	**<0.001**
Handwashing before eating						
No	1	1	1	1		
Yes	**0.11 (0.04, 0.30)**	**0.15 (0.03, 0.71)**	1.08 (0.25, 4.68)	0.79 (0.17, 3.68)	0.051	0.236

D. Urticarial rash

Factor	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	Interaction p	Interaction p**
Age	1.02 (0.99, 1.04)	1.01 (0.99, 1.04)	1.03 (1.02, 1.03)	1.03 (1.02, 1.04)	0.512	0.501

Sex						
Male	1	1	1	1		
Female	0.98 (0.54, 1.79)	1.07 (0.55, 2.10)	1.12 (0.85, 1.48)	1.23 (0.84, 1.80)	0.398	0.292
Maternal tribe						
Central Uganda	1	1	1	1		
Other, Ugandan	0.77 (0.40, 1.49)	0.80 (0.39, 1.65)	1.58 (0.98, 2.54)	1.65 (0.97, 2.79)		
Non-Ugandan	1.18 (0.35, 4.03)	1.15 (0.29, 4.55)	1.74 (0.97, 3.11)	**2.07 (1.16, 3.69)**	0.970	0.997
Paternal tribe						
Central Uganda	1	1	1	1		
Other, Ugandan	1.08 (0.55, 3.28)	1.03 (0.42, 2.52)	**0.43 (0.29, 0.65)**	**0.43 (0.27, 0.68)**		
Non-Ugandan	1.44 (0.63, 3.28)	1.28 (0.34, 4.80)	0.62 (0.35, 1.08)	0.44 (0.23, 0.84)	0.193	0.411
Maternal history of allergies						
No	1	1	1	1		
Yes	2.29 (0.83, 6.32)	2.43 (0.84, 7.03)	**2.19 (1.50, 3.21)**	**2.23 (1.51, 3.30)**	0.685	0.513
Paternal history of allergies						
No	1	1	1	1		
Yes	2.19 (0.84, 5.70)	1.62 (0.68, 3.87)	0.79 (0.39, 1.59)	0.90 (0.46, 1.76)	0.096	0.122
Malaria treatment, last 12 months						
No	1	1	1	1		
Yes	1.25 (0.78, 2.01)	1.21 (0.71, 2.07)	**1.69 (1.25, 2.30)**	**1.69 (1.14, 2.51)**	0.149	0.165
HIV						
Negative	1	1	1	1		
Positive	3.19 (0.80, 12.7)	2.67 (0.64, 11.1)	1.05 (0.63, 1.77)	1.02 (0.62, 1.69)	0.595	0.391
E. Rhinitis						
Factor	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	OR (95% CI)	OR (95% CI) – *Additionally adjusted for Sm (CCA)**	Interaction p	Interaction p**
Age	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)	1.02 (1.00, 1.03)	1.02 (1.00, 1.04)	0.576	0.597
Sex						

Male	1	1	1	1		
Female	1.31 (0.71, 2.40)	1.38 (0.69, 2.73)	1.42 (0.69, 2.88)	1.48 (0.72, 3.05)	0.786	0.212
Older siblings (Yes/No)						
No	1	1	1	1		
Yes	1.72 (0.73, 4.08)	1.67 (0.71, 3.96)	**0.63 (0.46, 0.86)**	**0.61 (0.42, 0.88)**	0.098	**0.040**
Younger siblings (Yes/No)						
No	1	1	1	1		
Yes	1.59 (0.80, 3.15)	1.58 (0.83, 2.99)	2.07 (0.67, 6.41)	1.81 (0.59, 5.53)	0.859	0.740
Location of birth						
City	1	1	1	1		
Town	0.09 (0.02, 5.55)	0.08 (0.00, 2.48)	**0.50 (0.29, 0.85)**	0.66 (0.29, 1.49)		
Village	0.29 (0.03, 3.05)	0.22 (0.03, 1.68)	**0.28 (0.12, 0.64)**	0.42 (0.15, 1.19)	0.135	0.241
Maternal history of allergies						
No	1	1	1	1		
Yes	**3.56 (1.28, 9.93)**	**3.95 (1.41, 11.1)**	1.58 (0.85, 2.96)	1.69 (0.96, 2.97)	0.676	0.878
Paternal history of allergies						
No	1	1	1	1		
Yes	**3.29 (1.24, 8.76)**	**3.56 (1.28, 9.93)**	1.30 (0.61, 2.76)	1.15 (0.48, 2.79)	0.435	0.264
Hand washing after toilet						
No	1	1	1	1		
Yes	2.26 (0.68, 7.47)	2.54 (0.81, 7.95)	1.91 (0.92, 3.94)	1.91 (0.85, 4.32)	0.212	0.221
HIV						
Negative	1	1	1	1		
Positive	3.15 (1.18, 8.39)	2.48 (0.59, 10.4)	1.17 (0.57, 2.40)	1.34 (0.66, 2.72)	0.029	0.228

Table shows only non-helminth-related factors that were associated with SPT reactivity, asIgE sensitisation, wheeze, urticaria and rhinitis in either the urban or the rural survey. All other non-helminth-related factors that were assessed are listed in the statistical methods section. Significant associations are highlighted in bold.

* Adjusting for other *Sm* infection variables (KK / PCR), *Sm* infection intensity (KK) and SWA- and SEA-specific antibodies paints a similar picture.

** Additionally adjusted for Sm infection (CCA)

CCA: Circulating Cathodic Antigen.

Table S5. Crude and adjusted associations with IgE sensitisation (ImmunoCAP IgE > 0.35 kU/L) to any of *D. pteronyssinus*, *A. hypogaea* or *B. germanica*

	UNADJUSTED ANALYSIS						ADJUSTED ANALYSIS				
		URBAN			**RURAL**		**URBAN**		**RURAL**		
Factor	N (%)≠	OR (95% CI)¶	p	N (%)≠	OR (95% CI)¶	p	OR (95% CI) ¶#	p	OR (95% CI)¶§	p	Interaction p
Age		0.99 (0.98, 1.01)	0.400		1.00 (0.99, 1.02)	0.076	0.99 (0.98, 1.01)	0.547	1.01 (0.98, 1.03)	0.589	0.728
Sex											
Male	47 (48)	1		241 (64)	1		1		1		
Female	101 (41)	0.75 (0.50, 1.10)	0.136	196 (49)	**0.56 (0.36, 0.86)**	**0.011**	0.77 (0.51, 1.15)	0.200	0.69 (0.42, 1.14)	0.140	0.407
Younger siblings (Yes/No)											
No	27 (33)	1		61 (62)	1		1		1		
Yes	106 (46)	1.76 (0.96, 3.25)	0.065	313 (56)	0.81 (0.57, 1.14)	0.213	**2.07 (1.07, 4.01)**	**0.030**	0.76 (0.53, 1.09)	0.129	**0.008**
Occupation											
Student or child (not at school)	68 (48)	1		64 (51)	1		1		1		
Unemployed or housewife	34 (40)	0.70 (0.42, 1.16)		58 (54)	1.23 (0.75, 2.01)		0.70 (0.31, 1.60)		**2.05 (1.38, 3.03)**		
Agricultural, fishing or lake related	4 (36)	0.61 (0.17, 2.12)		251 (61)	**1.58 (1.12, 2.24)**		0.56 (0.13, 2.46)		**1.87 (1.04, 3.37)**		
Professional or service providers	27 (38)	0.64 (0.37, 1.11)	0.391	61 (47)	0.78 (0.49, 1.25)	**0.020**	0.61 (0.26, 1.43)	0.725	1.38 (0.72, 2.66)	**0.014**	0.148
Lake contact											
Never	39 (45)	1					1				
Rarely	69 (42)	0.89 (0.49, 1.62)		8 (42)	1		0.98 (0.52, 1.85)		1		
Once a month	11 (35)	0.67 (0.36, 1.26)					0.74 (0.38, 1.41)				
Once a week	14 (50)	1.23 (0.47, 3.19)	1.000	25 (44)	0.93 (0.29, 3.00)		1.32 (0.49, 3.55)	0.835	0.82 (0.23, 2.89)		
Daily/ almost daily				343 (59)	2.22 (0.63, 7.86)	**0.034**			1.64 (0.45, 5.90)	0.174	
Bathe in water from lake?											
No	121 (42)	1		15 (63)	1		1		1		
Yes	12 (57)	1.85 (0.51, 6.66)	0.327	361 (57)	0.49 (0.19, 1.27)	0.139	1.86 (0.50, 6.87)	0.331	0.42 (0.15, 1.11)	0.078	0.065
Hand washing after toilet											
No	15 (39)	1		148 (71)	1		1		1		
Yes	117 (43)	1.16 (0.61, 2.23)	0.627	228 (50)	**0.37 (0.25, 0.55)**	**<0.001**	1.36 (0.69, 2.66)	0.344	**0.43 (0.30, 0.61)**	**<0.001**	**0.003**
SWA-specific IgE*		2.54 (0.56, 11.4)	0.209		**4.15 (2.05, 8.44)**	**<0.001**	2.95 (0.51, 17.2)	0.214	**6.17 (2.79, 13.6)**	**<0.001**	0.459

SWA-specific IgG4*		1.01 (0.86, 1.18)	0.898		**1.22 (1.02, 1.45)**	**0.032**	1.01 (0.85, 1.19)	0.932	1.07 (0.89, 1.27)	0.470	0.433
SEA-specific IgG4*		1.10 (0.98, 1.24)	0.090		**1.14 (1.02, 1.26)**	**0.023**	1.11 (0.97, 1.25)	0.102	1.07 (0.95, 1.21)	0.227	0.704
SWA-specific IgG*		2.74 (0.99, 7.59)	**0.052**		**3.04 (1.82, 5.08)**	**<0.001**	**3.33 (1.12, 9.86)**	**0.031**	1.53 (0.82, 2.86)	0.177	0.340
SEA-specific IgG*		1.58 (0.63, 4.01)	0.310		**2.41 (1.57, 3.66)**	**<0.001**	1.77 (0.63, 4.96)	0.260	1.43 (0.88, 2.30)	0.138	0.796
S. mansoni infection (KK)											
Uninfected	119 (44)	1		271 (55)	1		1		1		
Infected	6 (43)	0.95 (0.29, 3.09)	0.931	118 (63)	**1.39 (1.02, 1.90)**	**0.036**	1.06 (0.34, 3.35)	0.910	**1.52 (1.19, 1.94)**	**0.002**	0.180
S. mansoni infection intensity (KK)											
Uninfected	119 (44)	1		271 (55)	1		1		1		
Light	2 (29)	0.51 (0.08, 3.09)		54 (57)	1.23 (0.85, 1.78)		0.66 (0.11, 4.04)		**1.74 (1.18, 2.54)**		
Moderate	2 (50)	1.26 (0.15, 10.8)		35 (66)	1.23 (0.65, 2.32)		1.20 (0.16, 8.79)		0.94 (0.46, 1.90)		
Heavy	2 (67)	2.54 (0.13, 49.7)	0.711	29 (73)	2.44 (0.93, 6.38)	**0.019**	2.32 (0.13, 40.9)	0.662	2.37 (0.71, 7.83)	**0.028**	0.536
Any nematode infection**											
No	109 (42)	1		281 (54)	1		1		1		
Yes	16 (62)	2.22 (0.83, 5.94)	0.108	108 (66)	**1.90 (1.29, 2.81)**	**0.002**	2.34 (0.76, 7.19)	0.130	1.53 (0.94, 2.49)	0.084	0.287
Slept under mosquito net last night?											
No	35 (45)	1		203 (62)	1		1		1		
Yes	97 (42)	0.88 (0.49, 1.59)	0.678	172 (52)	**0.62 (0.41, 0.94)**	**0.025**	0.93 (0.52, 1.66)	0.958	**0.63 (0.41, 0.97)**	**0.037**	0.316
Malaria treatment, last 12months											
No	83 (45)	1		202 (63)	1		1		1		
Yes	48 (39)	0.78 (0.45, 1.34)	0.352	221 (51)	**0.53 (0.36, 0.75)**	**0.001**	0.78 (0.46, 1.35)	0.365	**0.52 (0.34, 0.81)**	**0.005**	0.185

Table shows only factors that were associated with IgE sensitisation (before and/or after adjustment) in either the urban or the rural survey. All other factors that were assessed are listed in the methods section. Significant associations are highlighted in bold. Interaction p values are shown to denote whether tests for interaction, using the adjusted model, showed statistical evidence for urban-rural differences in associations with IgE sensitisation, or not.

≠Number (percentage in parenthesis) of IgE sensitised individuals in each category

¶Odds ratios (ORs) and 95% confidence intervals (CI) adjusted for survey design

§All ORs were adjusted for hand washing after toilet use, mosquito net use, malaria treatment, age and sex.

#All ORs were adjusted for age and sex.

*Log_{10} (concentration+1) transformation applied before analysis

**Infection with any of *Ascaris lumbricoides, Trichuris trichiura* (assessed by KK), *Necator americanus, Strongyloides stercoralis* (assessed by PCR).

KK: Kato-Katz; SWA: *Schistosoma* adult worm antigen; SEA: *Schistosoma* egg antigen.

Table S6. Summary of risk factors for IgE sensitisation (ImmunoCAP IgE > 0.35 kU/L) to individual allergen extracts

	URBAN SURVEY		RURAL SURVEY	
Outcome	**Factors positively associated with outcome**	**Factors inversely associated with outcome**	**Factors positively associated with outcome**	**Factors inversely associated with outcome**
D. pteronyssinus-specific IgE sensitisation (ImmunoCAP)	• Presence of younger siblings (p=0.020) • Paternal history of allergies (p=0.041) • Maternal history of allergies (p=0.034)	-	• *T. trichiura* infection (p<0.001) • SWA-specific IgG (p=0.016) • SEA-specific IgG (p=0.037) • Maternal history of allergies (p=0.004) • Age (p=0.036) • engaging in agricultural / fishing / lake related activities or being unemployed (p=0.043)	• Hand washing after toilet use (p=0.008) • Bathing in lake water (p<0.001) • Presence of younger siblings (p=0.008) • Female gender (p=0.001) • Mosquito net use (p=0.052)
B. germanica-specific IgE sensitisation (ImmunoCAP)	• SEA-specific IgE (p=0.034) • Presence of younger siblings (p=0.045)	• *S. mansoni* infection (CCA) [p=0.024]	• SWA-specific IgE (p<0.001) • Any nematode infection (p=0.063) • *Sm* infection (KK) [p=0.060]	• Hand washing after toilet use (p=0.001) • Bathing in lake water (p=0.01) • Malaria infection (p=0.01) • Recent malaria treatment (p=0.010)

A. hypogaea-specific IgE sensitisation (ImmunoCAP)	• SEA-specific IgE (p=0.051) • Hookworm infection (p=0.031)	-	• SEA-specific IgE (p<0.001) • BCG scar (p=0.013) • Worm treatment during pregnancy (p=0.002)	• Recent malaria treatment (p=0.004)
D. pteronyssinus-specific IgE (ImmunoCAP) – continuous variable	-	-	• SWA-specific IgE (p=0.004), IgG (p=0.027) • SEA-specific IgG (p=0.035) • *S. mansoni* infection (KK) (p=0.029) • Paternal history of allergies (p=0.028)	• Female gender (p<0.001) • Recent malaria treatment (p=0.029) • Bathing in lake water (p=0.023)
B. germanica-specific IgE sensitisation (ImmunoCAP)-continuous variable	• SWA-specific IgG (p=0.011) • SEA-specific IgE (p=0.001)	-	• SWA-specific IgE (p<0.001) and IgG (p=0.008) • SEA-specific IgG (p=0.027) • *A. lumbricoides* infection (p<0.001)	• Hand washing (before eating [p=0.040], after toilet [p=0.002]) • Bathing in lake water (p=0.008) • Malaria infection (p<0.001) • Recent malaria treatment (p=0.005) • Female gender (p=0.004),

A. hypogaea-specific IgE sensitisation (ImmunoCAP)-continuous variable	• *S. mansoni* infection [PCR] (p=0.008) • *S. stercoralis* infection (p<0.001) • Hookworm infection (p=0.003) • Any nematode infection (p=0.002) • SEA-specific IgE (p=0.002), IgG4 (p<0.001) and IgG (p=0.025) and IgG4 (p=0.001) • SWA-specific IgG (p=0.003) • Presence of younger siblings (p=0.034)	-	• SWA-specific IgE (p<0.001), IgG (p<0.001), • SEA-specific IgE (p=0.001), IgG (p<0.001), • Hookworm infection (p=0.017)	• Female gender (p=0.011) • Recent malaria treatment (p=0.002) • Bathing in lake water (p=0.005) • Malaria infection (p=0.043) • Hand washing after toilet use (p=0.028)

P values are from adjusted analyses, conducted as described in the main text. The following were assessed as potential risk factors: age, sex, presence of older/younger siblings, maternal tribe, paternal tribe, location of birth, occupation, frequency of lake contact, type of bathing water, hand-washing behaviour [before eating, after toilet use], footwear outside the house, smoking, alcohol use, helminth infections, exposure to anthelminthic treatment in utero, anthelminthic treatment in last 12 months, maternal and paternal history of allergy/eczema/asthma, BCG scar, immunization history, treatment for malaria in last 12 months, malaria infection, HIV infection and plasma concentration of SEA- and SWA-specific IgE, IgG4 and IgG antibodies.

Table S7. Crude and adjusted associations with clinical allergy-related outcomes

		UNADJUSTED ANALYSIS					ADJUSTED ANALYSIS				
	URBAN			RURAL			URBAN		RURAL		
WHEEZE (AGE≥ 5 YEARS)	N (%)≠	OR (95% CI)[a]	p	N (%)≠	OR (95% CI)[a]	p	OR (95% CI)[a,b]	p	OR (95% CI)[a,c]	p	Interaction p
Age		1.02 (0.99, 1.03)	0.067		**1.02 (1.01, 1.04)**	**0.001**	1.01 (0.99, 1.02)	0.240	**1.00 (1.00, 1.03)**	**0.003**	0.386
Sex											
Male	8 (2)	1		56 (4)	1		1		1		
Female	16 (2)	1.19 (0.59, 2.42)	0.602	31 (2)	**0.45 (0.29, 0.69)**	**0.001**	1.30 (0.55, 3.07)	0.531	**0.47 (0.31, 0.73)**	**0.002**	0.087
Older siblings (Yes/No)											
No	11 (3)	1		20 (4)	1		1		1		
Yes	13 (2)	**0.46 (0.24, 0.89)**	**0.023**	62 (4)	0.84 (0.39, 1.77)	0.642	**0.36 (0.19, 0.73)**	**0.006**	0.88 (0.43, 1.79)	0.706	0.177
Occupation											
Student or child (not at school)	12 (3)	1		6 (1)	1		1		1		
Unemployed or housewife	7 (2)	0.88 (0.51, 1.54)		8 (3)	1.24 (0.36, 4.22)		0.46 (0.15, 1.41)		1.68 (0.45, 6.31)		
Agricultural, fishing or lake related	2 (3)	1.26 (0.32, 4.94)		61 (4)	**4.35 (1.38, 13.6)**		0.44 (0.03, 6.96)		3.85 (0.96, 15.4)		
Professional or service providers	3 (1)	0.35 (0.07, 1.55)	0.563	12 (3)	3.10 (0.86, 11.1)	**0.035**	**0.08 (0.01, 0.77)**	0.194	3.75 (0.79, 17.6)	0.258	0.051
Maternal history of allergies											
No	15 (2)	1		59 (3)	1		1		1		
Yes	7 (7)	**4.18 (1.65, 10.6)**	**0.004**	11 (3)	0.95 (0.38, 2.38)	0.926	3.34 (0.82, 13.6)	0.089	1.11 (0.45, 2.71)	0.818	0.083
Paternal history of allergies											
No	14 (2)	1		55 (3)	1		1		1		
Yes	5 (9)	**5.73 (1.34, 24.5)**	**0.021**	9 (4)	2.11 (0.96, 4.61)	0.061	4.96 (0.81, 30.4)	0.080	**2.32 (0.97, 5.49)**	**0.056**	0.259
Paternal tribe											
Central Uganda	8 (2)	1		38 (3)	1		1		1		
Other, Ugandan	12 (2)	1.41 (0.50, 3.96)		39 (3)	0.68 (0.38, 1.20)		1.58 (0.35, 7.16)		0.66 (0.38, 1.18)		
Non-Ugandan, African	4 (4)	**2.66 (1.14, 6.19)**	0.061	9 (3)	0.65 (0.36, 1.15)	0.193	**4.25 (1.94, 9.34)**	**0.001**	0.61 (0.36, 1.15)	0.173	**<0.001**
Hand washing before eating											
No	1 (1)	1		3 (3)	1		1		1		
Yes	23 (2)	**0.17 (0.05, 0.54)**	**0.004**	79 (4)	0.82 (0.19, 3.51)	0.785	**0.11 (0.04, 0.30)**	**<0.001**	1.08 (0.25, 4.68)	0.914	0.051

SWA-specific IgG*		0.21 (0.02, 2.18)	0.183		**27.4 (4.6, 162.3)**	**0.001**	**0.13 (0.02, 0.81)**	**0.030**	**26.8 (4.6, 158.6)**	**0.001**	**<0.001**
SEA-specific IgG*		**0.24 (0.06, 0.97)**	**0.046**		**4.87 (1.09, 21.6)**	**0.038**	**0.15 (0.05, 0.54)**	**0.005**	**5.91 (1.07, 32.4)**	**0.041**	**0.001**
SWA-specific IgG4*		**1.28 (1.02, 1.63)**	**0.037**		4.53 (0.84, 24.4)	0.076	1.46 (0.98, 2.19)	0.060	5.38 (0.73, 39.9)	0.096	0.276
Any nematode infection											
No	16 (2)	1		60 (4)	1		1		1		
Yes	1 (1)	0.51 (0.06, 4.22)	0.513	12 (2)	0.45 (0.19, 1.05)	0.064	0.92 (0.10, 8.27)	0.937	**0.41 (0.18, 0.97)**	**0.042**	0.744
URTICARIAL RASH											
	N (%)≠	OR (95% CI)[a]	p	N (%)≠	OR (95% CI)[a]	p	OR (95% CI)[a,d]	p	OR (95% CI)[a,e]	p	Interaction p
Age		1.02 (0.99, 1.04)	0.059		**1.03 (1.02, 1.04)**	**<0.001**	1.02 (0.99, 1.04)	0.179	**1.03 (1.02, 1.03)**	**<0.001**	0.512
Sex											
Male	20 (4)	1		160 (9)	1		1		1		
Female	33 (4)	1.09 (0.64, 1.88)	0.734	174 (11)	1.21 (0.94, 1.54)	0.132	0.98 (0.54, 1.79)	0.956	1.12 (0.85, 1.48)	0.399	0.398
Occupation											
Student or child (not at school)	19 (3)	1		74 (6)	1		1		1		
Unemployed or housewife	13 (4)	1.57 (0.63, 3.91)		45 (15)	**2.92 (1.86, 4.56)**		0.74 (0.36, 1.52)		1.49 (0.73, 3.00)		
Agricultural, fishing or lake related	5 (8)	3.13 (0.93, 10.5)		162 (12)	**2.45 (1.68, 3.56)**		0.43 (0.04, 4.56)		1.25 (0.68, 2.28)		
Professional or service providers	16 (5)	1.76 (0.85, 3.63)	0.154	53 (11)	**2.14 (1.49, 3.06)**	**<0.001**	1.25 (0.33, 4.76)	0.723	0.91 (0.48, 1.70)	0.674	0.595
Maternal tribe											
Central Uganda	22 (4)	1		100 (8)	1		1		1		
Other, Ugandan	23 (4)	1.04 (0.59, 1.84)		158 (10)	1.22 (0.84, 1.78)		0.77 (0.40, 1.49)		1.58 (0.98, 2.54)		
Non-Ugandan, African	8 (8)	1.92 (0.85, 4.32)	0.254	72 (14)	**1.61 (1.17, 2.22)**	**0.017**	1.18 (0.35, 4.03)	0.737	1.74 (0.97, 3.11)	0.091	0.970
Paternal tribe											
Central Uganda	22 (4)	1		158 (12)	1		1		1		
Other, Ugandan	25 (4)	1.08 (0.54, 2.18)		127 (8)	**0.69 (0.55, 0.88)**		1.08 (0.55, 3.28)		**0.43 (0.29, 0.65)**		
Non-Ugandan, African	6 (5)	1.41 (0.65, 3.04)	0.644	49 (12)	0.98 (0.71, 1.37)	**0.013**	1.44 (0.63, 3.28)	0.625	0.62 (0.35, 1.08)	**0.001**	0.193
Maternal history of allergies											
No	36 (3)	1		206 (9)	1		1		1		
Yes	11 (9)	**2.68 (1.26, 5.72)**	**0.013**	70 (13)	**1.82 (1.31, 2.52)**	**0.001**	2.29 (0.83, 6.32)	0.103	**2.19 (1.50, 3.21)**	**<0.001**	0.566
Paternal history of allergies											
No	36 (3)	1		235 (9)	1		1		1		
Yes	6 (9)	**2.72 (1.05, 6.98)**	**0.039**	31 (10)	0.97 (0.52, 1.83)	0.946	2.19 (0.84, 5.70)	0.102	0.79 (0.39, 1.59)	0.899	0.096

SEA-specific IgE*		0.89 (0.55, 1.44)	0.630		2.13 (0.81, 5.61)	0.122	0.72 (0.36, 1.46)	0.348	**2.83 (1.44, 5.55)**	**0.004**	**0.022**
Bathe in water from lake?											
No	47 (4)	1		6 (8)	1		1		1		
Yes	6 (5)	1.40 (0.67, 2.89)	0.343	264 (12)	**3.03 (1.26, 7.24)**	**0.015**	0.87 (0.32, 2.37)	0.776	3.44 (0.09, 118)	0.479	0.147
Worm treatment, last 12 months											
No	13 (3)	1		18 (5)	1		1		1		
Yes	40 (5)	**1.98 (1.17, 3.35)**	**0.013**	316 (11)	**2.04 (1.40, 2.96)**	**0.001**	**2.11 (1.11, 4.01)**	**0.024**	1.40 (0.71, 2.77)	0.171	0.248
Worm treatment in pregnancy											
No	8 (4)	1		11 (11)	1		1		1		
Yes	16 (3)	0.71 (0.22, 2.24)	0.542	6 (5)	**0.25 (0.07, 0.86)**	**0.030**	1.02 (0.32, 3.26)	0.975	0.29 (0.07, 1.14)	0.074	0.508
Malaria treatment, last 12 months											
No	29 (4)	1		90 (7)	1		1		1		
Yes	23 (5)	1.28 (0.81, 2.05)	0.278	240 (12)	**1.68 (1.22, 2.32)**	**0.002**	1.25 (0.78, 2.01)	0.329	**1.69 (1.25, 2.30)**	**0.001**	0.149
HIV											
Negative	43 (4)	1		210 (11)	1		1		1		
Positive	5 (9)	2.49 (0.92, 6.79)	0.071	65 (16)	**1.55 (1.07, 2.22)**	**0.020**	3.19 (0.80, 12.7)	0.095	1.05 (0.63, 1.77)	0.829	0.595
RHINITIS	N (%)[≠]	OR (95% CI)[a]	p	N (%)[≠]	OR (95% CI)[a]	p	OR (95% CI)[a,f]	p	OR (95% CI)[a,g]	p	p (interaction)
Age		1.01 (0.99, 1.02)	0.102		**1.04 (1.03, 1.05)**	**<0.001**	0.99 (0.97, 1.01)	0.527	**1.02 (1.00, 1.03)**	**0.019**	0.576
Sex											
Male	14 (3)	1		44 (3)	**1**		1		1		
Female	31 (4)	1.48 (0.78, 2.80)	0.214	60 (4)	1.14 (0.61, 2.15)	0.668	1.31 (0.71, 2.40)	0.367	1.42 (0.69, 2.88)	0.320	0.786
Older siblings (Yes/No)											
No	9 (2)	1		30 (6)	1		1		1		
Yes	36 (4)	1.63 (0.88, 2.96)	0.109	69 (4)	**0.69 (0.52, 0.93)**	**0.016**	1.72 (0.73, 4.08)	0.204	**0.63 (0.46, 0.86)**	**0.005**	0.098
Younger siblings (Yes/No)											
No	7 (2)	1		8 (2)	1		1		1		
Yes	38 (4)	**2.34 (1.20, 4.55)**	**0.015**	91 (5)	1.91 (0.69, 5.23)	0.190	1.59 (0.80, 3.15)	0.171	2.07 (0.67, 6.41)	0.195	0.859
Occupation											
Student or child (not at school)	13 (2)	1		6 (1)	1		1		1		
Unemployed or housewife	19 (7)	**3.46 (1.48, 8.12)**		14 (5)	**6.21 (2.01, 19.1)**		4.75 (1.26, 17.9)		1.68 (0.23, 12.5)		

Agricultural, fishing or lake related	2 (3)	1.75 (0.35, 8.84)		58 (4)	**8.22 (2.76, 24.5)**		2.15 (0.14, 32.1)		2.60 (0.31, 22.1)		
Professional or service providers	11 (3)	1.76 (0.64, 4.83)	0.059	26 (6)	**8.34 (2.70, 25.7)**	**0.007**	3.17 (0.56, 17.8)	0.107	2.33 (0.25, 21.4)	0.407	0.259
Location of birth											
City	3 (6)	1		6 (10)	1		1		1		
Town	2 (1)	0.25 (0.03, 2.28)		14 (6)	**0.48 (0.28, 0.81)**		0.09 (0.02, 5.55)		**0.50 (0.29, 0.85)**		
Village	12 (4)	0.65 (0.22, 1.93)	0.456	75 (4)	**0.30 (0.14, 0.65)**	**0.009**	0.29 (0.03, 3.05)	0.496	**0.28 (0.12, 0.64)**	**0.011**	0.135
Maternal history of allergies											
No	28 (3)	1		68 (3)	1		1		1		
Yes	11 (9)	**3.48 (1.35, 8.99)**	**0.012**	18 (3)	1.07 (0.56, 2.04)	0.822	**3.56 (1.28, 9.93)**	**0.017**	1.58 (0.85, 2.96)	0.126	0.676
Paternal history of allergies											
No	32 (3)	1		70 (3)	1		1		1		
Yes	6 (9)	**3.07 (1.27, 7.39)**	**0.015**	10 (3)	0.79 (0.39, 1.58)	0.499	**3.29 (1.24, 8.76)**	**0.019**	1.30 (0.61, 2.76)	0.479	0.435
Lake contact											
Never	9 (2)	1					1				
Rarely	23 (4)	**2.15 (1.05, 4.44)**		6 (7)	1		1.35 (0.51, 3.61)		1		
Once a month	4 (3)	1.58 (0.43, 5.83)					0.55 (0.09, 3.18)				
Once a week	9 (7)	**3.95 (1.85, 8.42)**	**0.004**	11 (5)	1.01 (0.28, 3.62)		2.24 (0.82, 6.07)	0.364	1.29 (0.35, 4.68)		
Daily/ almost daily				82 (4)	0.84 (0.23, 3.03)	0.651			1.30 (0.33, 5.17)	0.807	
Bathe in water from lake?											
No	40 (3)	1		6 (8)	1		1		1		
Yes	5 (4)	1.36 (0.61, 3.05)	0.425	93 (4)	**0.42 (0.22, 0.79)**	**0.010**	0.89 (0.39, 2.07)	0.794	**0.45 (0.27, 0.78)**	**0.006**	0.223
Hand washing after toilet											
No	2 (1)	1		22 (3)	1		1		1		
Yes	43 (4)	**4.13 (1.06, 15.9)**	**0.041**	77 (5)	1.63 (0.85, 3.14)	0.135	2.26 (0.68, 7.47)	0.169	1.91 (0.92, 3.94)	0.078	0.212
HIV											
Negative	37 (3)	1		67 (3)	1		1		1		
Positive	5 (9)	**2.92 (1.22, 6.99)**	**0.019**	17 (4)	1.61 (0.80, 3.22)	0.172	**3.15 (1.18, 8.39)**	**0.023**	1.17 (0.57, 2.40)	0.659	**0.029**

Table shows only factors that were associated with clinical allergy-related outcomes in either the urban or the rural survey, before and/or after adjustment for confounding. All other factors that were assessed are listed in the statistical methods section. Significant associations are highlighted in bold. Interaction p values are shown to denote whether tests for interaction, using the adjusted model, showed statistical evidence for urban-rural differences in associations with wheeze / urticarial rash / rhinitis, or not.

≠Number (percentage in parenthesis) of individuals with respective allergic disease in each category

[a]Odds ratios (ORs) and 95% confidence intervals (CI) adjusted for survey design.

[b]ORs adjusted for hand washing before eating, maternal history of allergies, paternal history of allergies, presence of older siblings, age and sex.

[c]ORs adjusted for age and sex.

[d]ORs adjusted for maternal history of allergies, paternal history of allergies, alcohol use, age and sex.

[e]ORs adjusted for HIV infection status, maternal history of allergies, recent malaria treatment, paternal tribe, maternal tribe, age and sex.

[f]ORs adjusted for presence of younger siblings, maternal history of allergies, paternal history of allergies, hand washing after toilet use, HIV infection, age and sex.

[g]ORs adjusted for presence of older siblings, location of birth, age and sex.

*Log_{10} (concentration+1) transformation applied before analysis

SWA: *Schistosoma* adult worm antigen; SEA: *Schistosoma* egg antigen.

CHAPTER 5. ASSOCIATIONS BETWEEN ALLERGY-RELATED OUTCOMES AND HELMINTH- AND ALLERGEN EXTRACT-SPECIFIC ANTIBODY PROFILES

5.1 Preamble

This chapter uses questionnaire, clinical and laboratory data from the baseline survey of the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA) to map the association of total, helminth- and allergen extract-specific IgE and IgG4 profiles with helminth infection and allergy-related outcomes (**thesis objective 2**). Results are presented in Research paper 2 (below), titled "*Schistosoma mansoni*-specific immune responses and allergy in Uganda", published in *Parasite Immunology.* The focus was on infection with *Schistosoma mansoni,* rather than with other helminths, because of its high prevalence in the study setting.

This chapter also presents extra, unpublished results, showing associations between allergy-related outcomes and total, schistosome- and allergen extract-specific IgE and IgG4 profiles in 1) the outcome survey of the LaVIISWA trial (after three years of community-based intensive versus standard anthelminthic treatment), 2) the urban survey and 3) the asthma case-control study. Comparisons of antibody profiles are made between intensive and standard trial arms of the rural survey, between the urban and rural survey, and between asthmatic and non-asthmatic school children.

The chapter assesses the relationship between *Schistosoma*-specific antibody responses and allergy-related outcomes, seeking to elucidate antibody mechanisms underlying positive helminth-atopy associations and the low overall prevalence of clinical allergy in the present study settings. Specifically, the role of IgG4 and total IgE (as a proxy for polyclonally-stimulated IgE), relative to allergen-specific IgE, is explored.

5.2 Research paper 2: *Schistosoma mansoni*-specific immune responses and allergy in Uganda

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ORIGINAL ARTICLE

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Schistosoma mansoni-specific immune responses and allergy in Uganda

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Summary

Low allergy-related disease (ARD) prevalence in low-income countries may be partly attributed to helminth infections. In the *Schistosoma mansoni* (*Sm*)-endemic Lake Victoria islands (Uganda), we recently observed positive helminth-allergy associations, despite low ARD prevalence. To understand how *Sm*-induced cytokine and antibody profiles might influence allergic response profiles in this population, we assessed *Schistosoma* worm (SWA)- and egg antigen (SEA)-specific Th1 (IFN-γ), Th2 (IL-5, IL-13) and regulatory (IL-10) cytokine profiles (n = 407), and total (n = 471), SWA-, SEA- and allergen (house dust mite [HDM] and cockroach)-specific (as)IgE and IgG4 profiles (n = 2117) by ELISA. Wheeze was inversely associated with SWA-specific IFN-γ ($P < .001$) and IL-10 ($P = .058$), and SEA-specific IL-5 ($P = .004$). Conversely, having a detectable asIgE response was positively associated with SWA-specific IL-5 ($P = .006$) and IL-10 ($P < .001$). Total, SWA-, SEA- and allergen-specific IgE and IgG4 responses were higher among *Sm* Kato-Katz positive (SmKK+) and skin prick test (SPT)+ individuals compared to SmKK- and SPT- individuals. However, total and asIgG4/IgE ratios were lower among SPT+ and wheezing individuals. We conclude that, in this population, helminth-induced antibody and cytokine responses may underlie individual positive helminth-atopy associations, while the overall IgG4-IgE balance may contribute to the low overall prevalence of clinical allergies in such settings.

KEYWORDS
allergy, cytokine, ELISA, immunoglobulin, *Schistosoma* spp

1 | INTRODUCTION

Helminths have a small range of antigens that are strikingly homologous to common allergens.[1] These antigens induce immunoglobulin (Ig) E-mediated effector responses important for protection against helminth infection.[2,3] To survive in the host, helminths modulate this atopic pathway, and this may have a bystander protective effect against allergy-related disease (ARD).[4] While several animal and human studies provide compelling evidence of this protection,[5,6] others suggest that in some circumstances helminths may actually promote enhanced responses to allergens.[7,8]

Mechanisms underlying helminth-allergy associations in low-income countries (LICs) are not fully understood. Hypothesized pathways that underpin these associations are shown in Figure 1. Helminth-induced cytokine and antibody profiles may influence allergic responses and consequently epidemiological trends pertaining to ARDs.[5,9] Both helminth- and allergen-specific immune responses are characterized by elevated Th2-type responses (interleukin [IL]-4,

LaVIISWA trial team members are in Appendix 1.

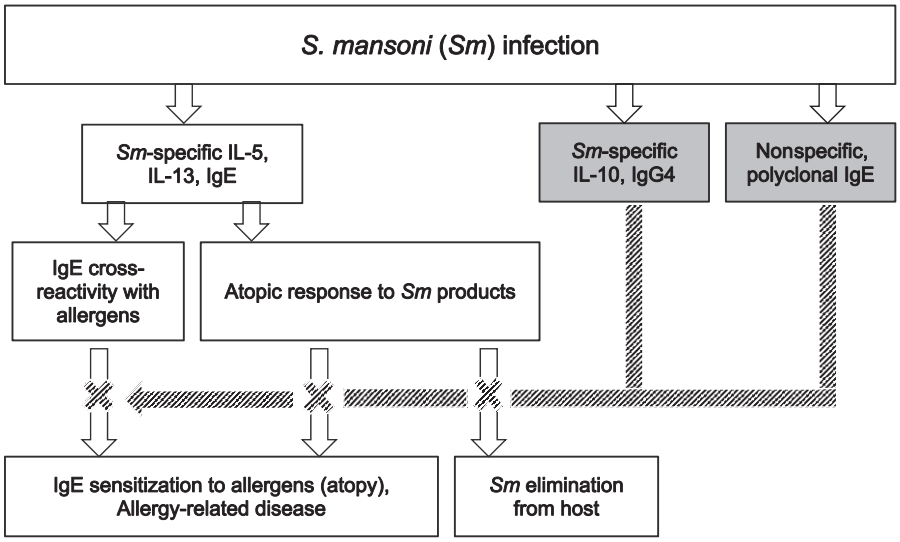

FIGURE 1 We hypothesize that the Th2 cytokine-induced *Sm*-specific IgE promotes potent, *Sm*-specific, atopic effector responses and *Sm* elimination from the host, and also cross-reactive responses to some allergens, resulting in positive *Sm*-allergy associations. By contrast, *Sm*-specific IL-10, IgG4 and/or nonspecific polyclonally stimulated IgE inhibit these allergy-related outcomes. White and shaded arrows denote promotion and inhibition, respectively

IL-5 and IL-13).[10,11] Helminths, unlike allergens, further induce strong immunoregulation epitomized by IL-10 production.[12] Typically, these cytokines influence the profile of antibodies involved in helminth infection and allergy. Helminth-induced IL-10 may drive immunoglobulin class switching to IgG4[13,14] which, akin to the Th2 cytokine-induced[15] polyclonally stimulated IgE, may inhibit development of allergen-specific effector responses,[5,16] leading to inverse helminth-allergy associations. Conversely, helminth-induced protein-specific IgE may promote strong, cross-reactive helminth- and allergen-specific responses, resulting in positive helminth-allergy associations.[17,18]

Emerging epidemiological data on helminth-allergy associations in Uganda reflect the complex interaction between helminths and allergens: while observational analyses in a birth cohort suggested a protective effect of childhood and maternal helminths against childhood eczema[19] that was reversed by maternal anthelminthic treatment,[20] we recently reported positive helminth-allergy associations in a survey conducted in the *Schistosoma mansoni* (*Sm*)-endemic Lake Victoria islands, albeit against a backdrop of low ARD prevalence.[21] To establish how *Sm*-induced cytokine and antibody profiles underpinned helminth-allergy associations in the above survey, we here describe an assessment of *Sm*-specific cytokine profiles, as well as total, allergen- and *Sm*-specific IgE and IgG4 profiles, and their relationship with *Sm* infection status, wheeze and atopy.

2 | METHODS

2.1 | Study population

Samples were collected during the baseline household survey preceding a cluster-randomized trial of standard vs intensive anthelminthic intervention (the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases, LaVIISWA; ISRCTN47196031) described elsewhere.[21,22] Briefly, each consenting LaVIISWA participant completed a questionnaire, provided blood, urine and stool and underwent skin prick testing (SPT). Primary allergy-related outcomes were reported wheeze in the previous 12 months and atopy. Wheeze is widely used as a surrogate for asthma in epidemiological studies[23] and was assessed for all ages using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Such symptom questionnaires have been identified as the best way to estimate asthma prevalence in epidemiological studies.[23] The ISAAC questionnaire was used to ask participants (or their caregivers) if they had ever wheezed and if so, if they had wheezed in the last 12 months. Details on aetiology were not collected. Atopy was defined as (i) SPT reactivity to any of *Dermatophagoides* mix, *Blomia tropicalis* or German cockroach (*Blattella germanica*) [ALK-Abelló; supplied by Laboratory Specialities Ltd., South Africa], and (ii) detectable IgE response (>312 ng/mL by ELISA[21]) to *Dermatophagoides pteronyssinus* [hereinafter "house dust mite (HDM)"] and/or German cockroach whole allergen extracts (Greer Labs, USA).

Ethics committees of Uganda Virus Research Institute, London School of Hygiene and Tropical Medicine and Uganda National Council for Science and Technology approved the study.

2.2 | Laboratory methods

Two slides from one stool sample per individual were independently examined by different technicians for *Sm* eggs using the Kato-Katz method.[24]

We assessed IFN-γ (Th1-type), IL-5, IL-13 (Th2-type) and IL-10 (regulatory) levels by ELISA using supernatants from six-day whole blood cultures stimulated with *Schistosoma* worm (SWA) and egg antigens (SEA), as previously described.[25] Briefly, heparinized blood was diluted with RPMI 1640 medium (Life technologies, UK) supplemented with penicillin, streptomycin, glutamine and Hepes buffer (all from Life technologies, UK), plated in 96-well culture plates and stimulated (at 37°C, 5% CO_2) with 10 μg/mL SWA or SEA (provided by Professor Mike Doenhoff, University of Nottingham) or mitogen (phytohaemagglutinin, PHA, Sigma, UK), or left unstimulated. Supernatants were harvested on day six and stored at −80°C until analysis. Cytokine

TABLE 1 Characteristics of participants

Characteristic	Survey population (N = 2316), n/N (%)	Immunological measure, n/N (%)		
		Cytokine responses[a] (N = 407)	Allergen-, SWA- and SEA-specific IgE and IgG4[b] (N = 2117)	Total IgE and IgG4[c] (N=471)
Age in years, median (IQR)	24 (8, 32)	9 (6, 16)	25 (10, 33)	19.5 (3, 31.25)
Male sex	1268/2316 (54.7)	168/407 (41.3)	1152/2117 (54.4)	225/471 (47.7)
PZQ in last 12 mo	382/2255 (16.9)	48/393 (12.2)	368/2062 (17.8)	15/459 (15.5)
Helminth infections				
S. mansoni (KK)	1041/1996 (51.4)	204/373 (54.7)	1008/1882 (53.6)	184/428 (42.9)
S. mansoni (urine CCA)	661/917 (72.0)	94/128 (73.4)	634/875 (72.5)	101/152 (66.5)
S. mansoni intensity (KK)				
Uninfected	995/1996 (48.6)	169/373 (45.3)	874/1882 (46.4)	244/428 (57.0)
Low	429/1996 (21.0)	77/373 (20.6)	411/1882 (21.8)	70/428 (16.4)
Moderate	288/1996 (13.7)	56/373 (15.0)	279/1882 (14.8)	51/428 (11.9)
Heavy	324/1996 (16.6)	71/373 (19.0)	318/1882 (16.9)	63/428 (14.7)
Any nematode infection[d]	788/2004 (39.3)	129/373 (34.6)	738/1889 (39.1)	87/428 (20.3)
Allergy-related outcomes				
Wheeze in last 12 mo	107/2301 (4.7)	14/404 (3.5)	106/2103 (5.04)	58/468 (12.4)
Atopy (SPT)				
Any	404/1976 (19.1)	78/372 (20.9)	403/1961 (20.6)	135/448 (30.1)
Dermatophagoides	190/1978 (9.0)	33/372 (8.9)	189/1963 (9.6)	61/448 (13.6)
Blomia	205/1976 (9.6)	31/372 (8.3)	204/1961 (10.4)	67/447 (14.9)
Cockroach	272/1977 (13.2)	61/372 (16.4)	272/1962 (13.9)	90/448 (20.1)
Atopy (detectable asIgE)				
Any	1685/2117 (79.6)	320/403 (79.4)	1685/2117 (79.6)	358/471 (76.0)
Dermatophagoides	1534/2115 (72.5)	278/403 (68.9)	1534/2115 (72.5)	326/471 (69.2)
Cockroach	886/2117 (41.9	183/403 (45.4)	886/2117 (41.9)	186/471 (39.5)

PZQ, Praziquantel treatment; KK, Kato-Katz; CCA, circulating cathodic antigen; SPT, skin prick test; SWA, *Schistosoma* worm antigen; SEA, *Schistosoma* egg antigen; asIgE: allergen-specific IgE.

[a]Assessed using samples from 1- to 17-year-olds, to allow comparison with related cellular immunology studies in an urban birth cohort (data not shown here).

[b]Assessed in all survey participants that had sufficient plasma sample stored.

[c]Samples randomly selected from individuals with antigen-specific antibody data.

[d]Infection with any of *Ascaris lumbricoides*, *Trichuris trichiura (assessed by KK)*, *Necator americanus*, *Strongyloides stercoralis (assessed by PCR)* and *Mansonella perstans (assessed by modified Knott's method)*.

levels in supernatants were measured by ELISA (Becton Dickinson, USA). The net response to each stimulus was calculated by subtracting the concentration in the unstimulated control well. Response values that were below the dynamic range of the assay and those that were negative after subtraction of the response in the unstimulated well were assigned a value of zero.

HDM and cockroach extract-specific IgE and IgG4 were measured in plasma using an in-house ELISA described previously.[20] Briefly, MICROLON® 96-well plates (Greiner bio-one, UK) were coated overnight at 4°C with 5 μg/mL HDM or cockroach allergens and twofold dilutions of human IgE (Calbiochem, Beeston, UK) or IgG4 (Sigma-Aldrich) standards. Plates were blocked at room temperature (RT) with 1% skimmed milk and incubated overnight at 4°C with plasma samples diluted 1/20 (IgE assay) or 1/40 (IgG4 assay) with 10% foetal bovine serum in PBS-Tween 20. Specific IgE or IgG4 was detected using biotinylated monoclonal mouse anti-human IgE or IgG4 (BD Pharmingen™) and a streptavidin-horseradish peroxidase conjugate (Mast Group Ltd, Bootle, UK). O-phenylenediamine (Sigma-Aldrich) was used as a substrate, and the reaction stopped with 2M sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgE or IgG4 concentrations (ng/mL) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA). Total, SWA- and SEA-specific IgE and IgG4 ELISAs were performed using similar in-house procedures (detailed in this article's supporting information).

2.3 | Statistical methods

Our hypothesized mode of action of *S. mansoni*-induced cytokines and antibodies on allergy-related outcomes is illustrated in Figure 1. Using STATA 13.1 (College Station, Texas, USA), we performed cross-sectional analyses to assess whether *Sm* Kato-Katz positivity and allergy-related outcomes were associated with antibody and cytokine levels, using the "svy" command to allow for the non–self-weighting cluster survey design. Raw cytokine and antibody responses were skewed, so $\log_{10}$ (concentration+1)-transformed antibody and cytokine data were used in our regression models; we back-transformed the results to obtain geometric mean ratios and 95% confidence intervals. Crude and age- and sex-adjusted analyses were performed. Associations between antibody responses were estimated using Spearman's correlation coefficient (r_s). We used a 5% significance level for all analyses. *P* values quoted in the main text are from adjusted analyses.

3 | RESULTS

Questionnaire data were obtained from 2316 participants.[22] Their characteristics and those of participants for whom cytokine and antibody responses were assessed are shown in Table 1. Participants for whom cytokine (n = 407) and total antibody levels (n = 471) were assessed were a subset of participants who had allergen-, SWA- and SEA-specific antibody results (n = 2117). Cytokine responses were assessed using samples from 1- to 17-year-olds, to allow comparison with related cellular immunology studies in an urban birth cohort (data not shown). Allergen-, SWA- and SEA-specific responses were assessed in all survey participants that had sufficient plasma sample stored.

3.1 | *S. mansoni*-specific cytokines and allergy-related outcomes

Individuals who tested positive for *Sm* by Kato-Katz (SmKK+) had higher geometric mean concentrations of SWA-specific type 2 and regulatory cytokines compared to SmKK- individuals (Table 2), but this was statistically significant only for IL-5 (*P* = .034). However, there was no dose-response relationship with infection intensity (Table S3A). SEA-specific responses were similar between SmKK+ and SmKK- individuals.

Wheeze was inversely associated with SWA-specific IFN-γ (*P* < .001) and IL-10 (*P* = .058; Table 2), and SWA-specific IL-10/IL-5 and SEA-specific IL-10/IFN-γ ratios (Table S1). Wheezing individuals also had lower mean SEA-specific cytokine responses, although statistical evidence for an inverse association was observed only for SEA-specific IL-5 (*P* = .004).

Conversely, there was a crude positive association between SPT positivity and SWA-specific IL-10 (*P* = .048; Table 2); and individuals with detectable allergen-specific (as)IgE had higher SWA-specific IL-5 (*P* = .006) and IL-10 responses (*P* < .001; Table 2) and higher SWA- and SEA-specific IL-10/IFN-γ and IL-5/IFN-γ ratios (Table S1).

3.2 | Antibody responses, *S. mansoni* Kato-Katz positivity and allergy

Kato-Katz positivity was strongly positively associated with SWA- and SEA-specific IgE and IgG4 (*P* < .001), with HDM-specific IgE (*P* = .006) and HDM- and cockroach-specific IgG4 (*P* < .001; Table 3 and supplementary Figure S1). However, correlations between *Sm* antigen-specific antibodies and allergen-specific antibodies were weak (r_s < .4, Table S2).

Kato-Katz positivity was also strongly positively associated with total IgE (*P* < .001) (Table 3 and Figure S1), which was in turn weakly correlated with SEA-specific IgE but moderately correlated with SWA-specific IgE (r_s = .31 and r_s = .51, respectively; Table S2). Similarly, total IgG4 (*P* < .001), total IgG4/total IgE ratios (*P* = .005) and total IgE/asIgE ratios (*P* < .05) were positively associated with Kato-Katz positivity. In addition, there was a general dose-response relationship between *S. mansoni* infection intensity and antibody responses (Table S3B).

Cockroach-specific IgE and total IgE were positively associated with cockroach SPT reactivity. HDM-specific IgE and IgG4, SWA- and SEA-specific IgE and total IgE, were all positively associated with HDM SPT reactivity (Table 3 and Figure S1). In contrast, cockroach SPT reactivity was inversely associated with total IgG4/total IgE ratios (*P* = .022), and HDM SPT reactivity with total IgE/HDM-specific IgE ratios (*P* < .001).

Associations between wheeze and antibody responses (Table 3 and Figure S1), when significant, were inverse. HDM IgG4/IgE ratios (*P* = .032), cockroach-specific IgE (*P* = .003) and cockroach-specific IgG4 (*P* = .001) were all inversely associated with wheeze.

4 | DISCUSSION

In this highly *Sm*-endemic setting, associations between wheeze and *Sm*-specific cytokines and antibodies, when significant, were inverse. However, SPT reactivity and detectable asIgE were positively associated with the same *Sm*-specific responses.

In this population, *Sm* exposure is almost universal, and infection much higher than indicated by Kato-Katz: urine assessment for *Sm* circulating cathodic antigen (CCA) indicated a prevalence of over 70%, compared to 51.4% prevalence by Kato-Katz.[22] Therefore, Kato-Katz negativity in many study participants was indicative of lighter (rather than absent) infection. This explains why, although SWA-specific Th2-type and regulatory cytokine responses were generally higher among SmKK+ individuals, only SWA-specific IL-5 reached significant levels, and why SEA-specific responses were similar between SmKK+ and SmKK- individuals. Further support for these observations comes from supplementary analysis (Table S4A), which shows that cytokine responses were similar between SmKK-CCA+ and SmKK+CCA± individuals.

TABLE 2 Associations between *S. mansoni*-specific cytokine levels and (i) *S. mansoni* infection status, (ii) reported wheeze and (iii) atopy (SPT reactivity and detectable allergen-specific IgE)

		Geometric mean[a]		Unadjusted		Adjusted for age and sex	
Antigen	Cytokine	SmKK- n = 169	SmKK+ n = 204	GMR (95% CI)[b]	*P* value	GMR (95% CI)[b]	*P* value
SWA	IFN-γ	1.16	1.13	1.06 (0.86, 1.30)	.542	1.05 (0.87, 1.28)	.531
	IL-5	**14.92**	**49.47**	**1.43 (1.13, 1.81)**	**.005**	**1.32 (1.02, 1.71)**	**.034**
	IL-13	7.01	17.56	1.20 (0.94, 1.54)	.132	1.15 (0.88, 1.48)	.282
	IL-10	3.99	11.58	1.21 (0.97, 1.51)	.084	1.16 (0.91, 1.48)	.207
SEA	IFN-γ	0.73	0.56	0.97 (0.81, 1.17)	.760	0.98 (0.82, 1.18)	.884
	IL-5	5.02	3.11	0.84 (0.59, 1.19)	.320	0.84 (0.58, 1.19)	.319
	IL-13	2.25	1.95	0.86 (0.71, 1.05)	.127	0.88 (0.73, 1.06)	.190
	IL-10	3.19	4.42	0.93 (0.78, 1.13)	.486	0.93 (0.76, 1.13)	.477
		No wheeze n = 390	**Wheeze n = 14**				
SWA	IFN-γ	**1.27**	**0.23**	**0.60 (0.45, 0.80)**	**.001**	**0.57 (0.44, 0.76)**	**<.001**
	IL-5	29.12	27.59	1.29 (0.72, 2.33)	.373	1.14 (0.63, 2.08)	.657
	IL-13	11.75	9.78	1.26 (0.65, 2.45)	.465	1.17 (0.58, 2.36)	.635
	IL-10	**7.91**	**2.03**	**0.69 (0.47, 1.01)**	**.059**	**0.66 (0.43, 1.02)**	**.058**
SEA	IFN-γ	0.66	0.33	0.83 (0.66, 1.04)	.101	0.83 (0.65, 1.05)	.121
	IL-5	**4.37**	**1.20**	**0.52 (0.33, 0.83)**	**.007**	**0.51 (0.33, 0.79)**	**.004**
	IL-13	2.25	0.96	0.75 (0.44, 1.25)	.256	0.76 (0.44, 1.33)	.327
	IL-10	4.34	0.59	0.70 (0.35, 1.38)	.295	0.71 (0.37, 1.36)	.291
		SPT− n = 294	**SPT+[c] n = 78**				
SWA	IFN-γ	1.03	1.73	1.15 (0.96, 1.36)	.115	1.13 (0.94, 1.34)	.178
	IL-5	29.23	39.73	1.15 (0.86, 1.52)	.330	1.02 (0.75, 1.38)	.897
	IL-13	13.54	10.32	0.99 (0.72, 1.36)	.961	0.92 (0.67, 1.26)	.596
	IL-10	**6.79**	**12.00**	**1.25 (1.00, 1.55)**	**.048**	1.21 (0.95, 1.54)	.126
SEA	IFN-γ	0.55	0.99	1.11 (0.82, 1.51)	.493	1.13 (0.83, 1.52)	.423
	IL-5	4.54	3.68	1.00 (0.70, 1.45)	.965	0.98 (0.67, 1.43)	.935
	IL-13	2.81	1.09	0.84 (0.56, 1.25)	.376	0.85 (0.58, 1.25)	.413
	IL-10	4.12	3.09	0.94 (0.76, 1.16)	.560	0.96 (0.75, 1.22)	.730
		Undetectable asIgE n = 83	**Detectable asIgE[d] n = 320**				
SWA	IFN-γ	1.73	1.11	0.87 (0.62, 1.24)	.444	0.86 (0.60, 1.23)	.396
	IL-5	**16.44**	**34.25**	**1.43 (1.16, 1.75)**	**.001**	**1.32 (1.09, 1.61)**	**.006**
	IL-13	9.75	12.66	1.09 (0.77, 1.55)	.599	1.04 (0.74, 1.47)	.806
	IL-10	**3.76**	**9.26**	**1.34 (1.18, 1.51)**	**<.001**	**1.30 (1.16, 1.46)**	**<.001**
SEA	IFN-γ	1.12	0.56	0.79 (0.56, 1.13)	.190	0.79 (0.57, 1.11)	.176
	IL-5	3.78	4.17	1.13 (0.81, 1.58)	.459	1.12 (0.82, 1.54)	.450
	IL-13	2.21	2.21	0.88 (0.65, 1.22)	.449	0.90 (0.67, 1.21)	.484
	IL-10	3.49	4.17	1.05 (0.92, 1.21)	.424	1.06 (0.93, 1.21)	.382

SmKK−, Kato-Katz negative result (*S. mansoni*), single stool sample; SmKK+, Kato-Katz positivity for *S. mansoni*, single stool sample; SPT, skin prick test; SWA, *Schistosoma* worm antigen; SEA, *Schistosoma* egg antigen; asIgE, allergen-specific IgE; GMR, geometric mean ratio; 95% CI, 95% confidence interval. *P* values ≤.05 are highlighted in bold.

[a]All cytokine concentrations in pg/mL.

[b]Geometric mean ratios and 95% confidence intervals adjusted for the survey design.

[c]SPT reactivity to any one of *Dermatophagoides* mix, *Blomia tropicalis* or *Blattella germanica*.

[d]Detectable IgE to either *Dermatophagoides pteronyssinus* or *Blattella germanica*.

TABLE 3 Associations between antibody (IgE and IgG4) levels and Kato-Katz positivity (*S. mansoni*), SPT reactivity and reported wheeze

Antigen	Antibody/antibody ratio	Geometric mean[a]		aGMR (95% CI)[bc]	*P* value
		SmKK−	*SmKK+*		
SWA	IgE	**1080**	**2433**	**1.54 (1.28, 1.84)**	**<.001**
	IgG4	**4031**	**27 355**	**3.71 (3.14, 4.37)**	**<.001**
SEA	IgE	**1412**	**1833**	**1.32 (1.15, 1.52)**	**<.001**
	IgG4	**18 962**	**241 763**	**5.51 (4.55, 6.67)**	**<.001**
House dust mite	IgE	**0.782**	**10.678**	**1.25 (1.07, 1.45)**	**.006**
	IgG4	**0.001**	**0.192**	**1.79 (1.51, 2.13)**	**<.001**
	IgG4/IgE ratio	0.002	0.033	1.18 (0.58, 2.41)	.629
Cockroach	IgE	18.8	19.2	1.00 (0.82, 1.22)	.989
	IgG4	**0.002**	**0.292**	**1.50 (1.34, 1.68)**	**<.001**
	IgG4/IgE ratio	0.001	0.027	1.32 (0.94, 1.85)	.110
	Total IgE	**969**	**3073**	**1.37 (1.22, 1.54)**	**<.001**
	Total IgG4	**51 453**	**233 745**	**1.94 (1.49, 2.52)**	**<.001**
	Total IgG4/total IgE ratio	**52.16**	**75.24**	**1.36 (1.11, 1.67)**	**.005**
	Total IgE/cockroach IgE ratio	**3.79**	**12.60**	**1.32 (1.06, 1.66)**	**.014**
	Total IgE/dust mite IgE ratio	**0.562**	**1.301**	**1.13 (1.02, 1.25)**	**.016**
		Cockroach SPT−	*Cockroach SPT+*		
SWA	IgE	1704	1894	1.12 (0.94, 1.32)	.173
	IgG4	12 860	14 155	1.04 (0.85, 1.28)	.675
SEA	IgE	1611	1876	1.12 (0.97, 1.29)	.092
	IgG4	84 831	101 778	1.08 (0.92, 1.27)	.319
Dust mite	IgE	**2.6**	**42.2**	**1.59 (1.35, 1.89)**	**<.001**
	IgG4	0.022	0.061	1.06 (0.87, 1.29)	.498
	IgG4/IgE ratio	**0.009**	**0.001**	**0.56 (0.36, 0.85)**	**.010**
Cockroach	IgE	**18.9**	**39.1**	**1.25 (1.08, 1.46)**	**.004**
	IgG4	**0.054**	**0.686**	1.15 (0.95, 1.39)	.129
	IgG4/IgE ratio	0.003	0.017	0.69 (0.47, 1.02)	.064
	Total IgE	**1462**	**2787**	**1.22 (1.05, 1.42)**	**.011**
	Total IgG4	90 643	126 688	0.84 (0.65, 1.07)	.163
	Total IgG4/total IgE ratio	**60.41**	**45.80**	**0.75 (0.58, 0.95)**	**.022**
	Total IgE/cockroach IgE ratio	6.07	9.22	1.01 (0.82, 1.25)	.894
	Total IgE/dust mite IgE ratio	0.849	0.868	0.93 (0.84, 1.03)	.140
		Dust mite SPT−	*Dust mite SPT+*		
SWA	IgE	**1667**	**2409**	**1.26 (1.00, 1.57)**	**.043**
	IgG4	13 088	12 565	1.04 (0.79, 1.36)	.744
SEA	IgE	**1623**	**1887**	**1.23 (0.99, 1.53)**	**.055**
	IgG4	85 471	102 026	1.20 (0.85, 1.68)	.271
Dust mite	IgE	**2.5**	**242.9**	**2.10 (1.57, 2.81)**	**<.001**
	IgG4	**0.020**	**0.245**	**1.41 (0.99, 1.99)**	**.052**
	IgG4/IgE ratio	0.009	0.001	0.49 (0.23, 1.04)	.064

(Continues)

All statistically significant associations between atopy and *Sm*-specific cytokine responses were positive. Associations with whole blood cytokine responses are best interpreted taking into account total cell counts, but these data were unavailable. However, atopy-antibody associations were also positive. Besides, these results mirror our previous epidemiological observations in this population, where *Sm* infection was positively associated with *Dermatophagoides*-specific IgE, and atopy-wheeze associations were stronger in the presence of *Sm* infection.[21]

TABLE 3 (Continued)

		Dust mite SPT−	*Dust mite SPT+*		
Cockroach	IgE	**19.9**	**33.7**	**1.21 (1.03, 1.43)**	**.024**
	IgG4	0.065	0.405	1.13 (0.85, 1.50)	.395
	IgG4/IgE ratio	0.004	0.013	0.71 (0.48, 1.04)	.075
	Total IgE	**1533**	**2802**	**1.24 (1.03, 1.49)**	**.025**
	Total IgG4	89 885	156 204	1.09 (0.86, 1.38)	.438
	Total IgG4/total IgE ratio	57.43	55.45	0.89 (0.72, 1.11)	.292
	Total IgE/cockroach IgE ratio	6.41	8.11	1.01 (0.82, 1.26)	.893
	Total IgE/dust mite IgE ratio	**0.937**	**0.517**	**0.81 (0.74, 0.89)**	**<.001**
		No wheeze	*Wheeze*		
SWA	IgE	1672	2627	1.15 (0.81, 1.64)	.425
	IgG4	12 753	14 802	1.05 (0.71, 1.54)	.808
SEA	IgE	1636	1547	0.93 (0.66, 1.29)	.662
	IgG4	81 698	145 978	1.16 (0.77, 1.73)	.449
House dust mite	IgE	2.8	26.6	1.35 (0.89, 2.04)	.148
	IgG4	0.007	0.008	0.85 (0.59, 1.25)	.407
	IgG4/IgE ratio	**0.011**	**0.001**	**0.45 (0.22, 0.93)**	**.032**
Cockroach	IgE	**21.2**	**2.9**	**0.69 (0.55, 0.87)**	**.003**
	IgG4	**0.021**	**0.006**	**0.65 (0.53, 0.82)**	**.001**
	IgG4/IgE ratio	0.004	0.002	1.14 (0.47, 2.74)	.760
	Total IgE	1630	1522	0.88 (0.72, 1.07)	.205
	Total IgG4	100 983	74 475	0.85 (0.61, 1.17)	.302
	Total IgG4/total IgE ratio	61.00	47.36	0.95 (0.77, 1.18)	.683
	Total IgE/cockroach IgE ratio	6.29	6.86	0.91 (0.78, 1.05)	.187
	Total IgE/dust mite IgE ratio	0.857	0.752	0.91 (0.77, 1.05)	.188

SWA, *Schistosoma* worm antigen; SEA, *Schistosoma* egg antigen; SmKK−, Kato-Katz negative result (*S. mansoni*), single stool sample; SmKK+, Kato-Katz positive result, single stool sample; aGMR, adjusted geometric mean ratio; 95% CI, 95% confidence interval. *P* values ≤0.05 are highlighted in bold.

[a]All antibody concentrations in ng/mL.

[b]All geometric mean ratios and 95% confidence intervals adjusted for survey design, age and sex.

[c]Geometric mean ratios and 95% confidence intervals for associations between antibody levels and SPT reactivity and wheeze were additionally adjusted for SmKK result.

Our results were unexpected in view of earlier findings from Gabon[9] which showed an inverse association between dust mite SPT and SWA-specific IL-10 (albeit we used whole blood cultures, compared to peripheral blood mononuclear cells in the Gabon study). However, although IL-10 is chiefly immunomodulatory,[26-28] it may also enhance IgE production in already IgE-switched B cells;[13] these may be abundant in individuals from this helminth-endemic setting. SWA- and SEA-specific IgE were weakly positively associated with HDM SPT reactivity, perhaps unsurprisingly, as helminth antigens may induce cross-reactive helminth- and allergen-specific IgE effector responses. Total serum IgE, elevated during helminth infection mainly due to increased synthesis of polyclonal IgE, has been proposed to inhibit allergic responses.[29,30] However, contrasting evidence links high serum IgE levels to increased expression of IgE receptors on human basophils,[31] and we show positive associations between total IgE and SPT reactivity to both cockroach and dust mite.

In keeping with the original hypothesis, associations between wheeze and cytokine and antibody responses, when significant, were inverse. Furthermore, total and allergen-specific IgG4/IgE ratios were mostly inversely associated with atopy, implying that the regulatory role of IgG4 against allergy might best be assessed relative to IgE. Also, lower total/asIgE ratios among HDM SPT+ individuals are consistent with the perception that high total/asIgE ratios may be protective against allergic responses, because nonspecific polyclonal IgE may compete with asIgE to saturate IgE receptors.[29]

One limitation of assessing helminth-allergy associations and underlying mechanisms in this population is the almost universal exposure to helminths, and lack of data on duration of infection. We also report a large number of statistical tests, so some apparently "significant" findings could have occurred by chance. As we anticipated that some of our measures might be correlated, we did not formally adjust for multiplicity, instead we focussed on patterns of association and consistency of results, and on biological plausibility with reference to other

findings. Another potential limitation is that wheeze was relatively rare in the study population, and hence, some of our comparison groups (such as the age group 1-17 years) had a low prevalence. Besides, reported wheeze could easily be misclassified in this population due to lack of a direct translation of "wheeze" in the native languages.[21]

Nonetheless, our results generally agree with our epidemiological observations in the same population,[21] where we found a very low prevalence of clinical allergies, despite positive helminth-atopy associations.

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DISCLOSURES

None.

AUTHOR CONTRIBUTIONS

AME conceived the main study. AME, RES and MN led the field and clinic teams. AME, GN and JK participated in the design of laboratory studies. GN, JK, BW and JN performed the experiments. GN and ELW analysed the results. GN wrote the manuscript, with all authors contributing to the interpretation of the results, and revision and approval of the final manuscript. GN is the guarantor of the article.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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APPENDIX

LaVIISWA trial team

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5.3 Supplementary information for Research paper 2 (available in the article's online repository at https://onlinelibrary.wiley.com/doi/10.1111/pim.12506)

5.3.1 Experimental methods

5.3.1.1 S. mansoni worm (SWA)- and egg (SEA)-specific IgE and IgG4 ELISA

All but the first 2 columns of 4HX Immulon (Thermo Scientific, NY, USA) 96-well plates were coated with 50µl of SWA [8 µg/ml] or SEA [2.4 µg/ml] (purchased from Professor Mike Doenhoff, University of Nottingham) in bicarbonate buffer (0.1M, pH 9.6). Two-fold dilutions of human IgE (Calbiochem, Beeston, UK) or IgG4 (Sigma-Aldrich) standard, diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4^0C. Plates were washed with phosphate-buffered saline (PBS)-tween 20 solution, blocked with 150µl of skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4^0C with 50µl of plasma samples diluted 1/20 with 10% fetal bovine serum in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates overnight at 4^0C with 0.5µg/ml of biotinylated monoclonal mouse anti-human IgE or IgG4 (BD Pharmingen™), followed by a 1 hour incubation with a streptavidin-Horseradish Peroxidase (strep-HRP) conjugate (Mast Group Ltd, Bootle, UK), diluted 1/3000 with assay buffer, at RT. Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped with 30µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgE or IgG4 concentrations (ng/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

5.3.1.2 Total IgE ELISA

96-well Maxisorp plates (VWR, U.S.A) were coated with 100µl of polyclonal rabbit anti human IgE (Agilent technologies, Dako, Denmark) diluted 1/1000 in bicarbonate buffer (0.1M, pH 9.6) and incubated overnight at 4^oC. Plates were then washed with phosphate-

buffered saline (PBS)-tween 20 solution and blocked with 120µl of PBS-bovine serum albumin (BSA) solution for 1 hour at room temperature (RT). Plasma samples (100µl) diluted 1/50 in assay buffer (0.1M Tris pH 7.5 + 0.05% Tween-20), the blank (assay buffer) and National Institute for Biological Standards and Control (NIBSC) international IgE standards were added to the plates and incubated for 1 hour at RT. Plates were then washed and incubated with 100µl of biotinylated goat anti-human IgE (Vector laboratories, U.S.A, 0.5mg/ml), diluted 1/1000 with assay buffer, for 1 hour at RT. After another washing step, the plates were incubated with 100µl of streptavidin alkaline phosphatase (Roche Life Science), diluted 1/3000 with assay buffer, for 30 minutes at RT. 4-nitrophenyl phosphate disodium salt hexahydrate (p-NPP), diluted in diethanolamine buffer (DEA, 0.1M), was added, followed by 20 minutes incubation at RT in the dark for development. Sodium hydroxide (3M, 100µl) was then added to stop the reaction. Plates were read at 405 nm using an ELISA reader. Results were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

5.3.1.3 Total IgG4 ELISA

96-well Maxisorp plates (VWR, U.S.A) were coated with purified mouse anti-human IgG4 (BD Pharmingen™) in bicarbonate buffer (0.1 M, pH 9.6) overnight at 4°C, and blocked for 1 hour with 3% skimmed milk in 1X PBS at room temperature (RT). Plates were then incubated with plasma samples (diluted 1/800 in 0.1M Tris pH 7.5 + 0.05% Tween-20) and IgG4 standards (Sigma Aldrich) for one hour. Antibody binding was detected by incubating the plates with mouse anti-human IgG4 conjugated to horseradish peroxidase (Invitrogen) for one hour, followed by a colour reaction with o-phenylenediamine (Sigma Aldrich). Reactions were stopped with 2M Sulphuric acid. Absorbance was measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgG4 concentrations (ng/ml) were interpolated from standard curves using a five-parameter

curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, U.S.A).

5.3.2 Supplementary tables and figures

Table S1. Associations between *S. mansoni*-specific cytokine ratios and i) *S. mansoni* infection status, ii) reported wheeze and iii) atopy (SPT reactivity and detectable allergen-specific IgE)

				Unadjusted		Adjusted for age and sex	
Antigen	**Ratio**	**Geometric mean†**		**GMR (95% CI)‡**	**p value**	**GMR (95% CI) ‡**	**p value**
		SmKK- n=169	SmKK+ n=204				
SWA	IL-10/ IFN-γ	3.29	10.46	1.23 (0.89, 1.71)	0.192	1.18 (0.85, 1.65)	0.297
	IL-10/ IL-5	0.26	0.23	0.91 (0.74, 1.11)	0.337	0.92 (0.74, 1.15)	0.456
	IL-10/ IL-13	0.56	0.65	0.95 (0.71, 1.29)	0.767	0.95 (0.67, 1.33)	0.745
	IL-5/ IFN-γ	**12.79**	**43.52**	**1.36 (1.03, 1.80)**	**0.028**	1.25 (0.94, 1.65)	0.120
	IL-13/ IFN-γ	6.01	15.45	1.11 (0.81, 1.53)	0.498	1.03 (0.75, 1.43)	0.824
SEA	IL-10/ IFN-γ	4.54	8.14	1.00 (0.75, 1.34)	0.985	0.97 (0.73, 1.29)	0.849
	IL-10/ IL-5	0.65	1.39	0.97 (0.72, 1.31)	0.853	0.96 (0.71, 1.32)	0.826
	IL-10/ IL-13	1.42	2.16	0.96 (0.72, 1.29)	0.807	0.95 (0.69, 1.32)	0.768
	IL-5/ IFN-γ	6.88	5.55	0.94 (0.69, 1.29)	0.723	0.91 (0.66, 1.25)	0.550
	IL-13/ IFN-γ	3.09	3.49	0.85 (0.67, 1.07)	0.175	0.85 (0.68, 1.05)	0.123
		No wheeze n=390	Wheeze n=14				
SWA	IL-10/ IFN-γ	6.22	8.82	0.99 (0.68, 1.42)	0.961	0.97 (0.61, 1.55)	0.902
	IL-10/ IL-5	**0.26**	**0.07**	**0.79 (0.69, 0.91)**	**0.001**	**0.83 (0.70, 0.97)**	**0.023**
	IL-10/ IL-13	0.66	0.21	0.78 (0.53, 1.15)	0.206	0.79 (0.52, 1.21)	0.267
	IL-5/ IFN-γ	**22.87**	**119.94**	**2.51 (1.14, 5.51)**	**0.024**	**2.25 (1.03, 4.96)**	**0.044**
	IL-13/ IFN-γ	9.23	42.54	2.21 (0.91, 5.43)	0.079	2.08 (0.85, 5.06)	0.100
SEA	IL-10/ IFN-γ	**6.78**	**1.78**	**0.52 (0.34, 0.80)**	**0.004**	**0.53 (0.37, 0.74)**	**0.001**
	IL-10/ IL-5	0.99	0.49	1.44 (0.48, 4.24)	0.493	1.49 (0.53, 4.21)	0.432
	IL-10/ IL-13	1.88	0.61	0.78 (0.53, 1.16)	0.212	0.79 (0.55, 1.15)	0.220
	IL-5/ IFN-γ	6.59	3.62	0.57 (0.32, 1.05)	0.069	0.55 (0.29, 1.02)	0.058
	IL-13/ IFN-γ	3.40	2.90	0.69 (0.46, 1.03)	0.072	0.69 (0.45, 1.06)	0.088
		SPT- n=294	SPT+§ n=78				
SWA	IL-10/ IFN-γ	6.68	6.85	0.97 (0.76, 1.25)	0.840	0.94 (0.73, 1.22)	0.639
	IL-10/ IL-5	0.22	0.32	1.06 (0.86, 1.30)	0.532	1.11 (0.92, 1.34)	0.276
	IL-10/ IL-13	0.48	1.22	1.28 (0.94, 1.74)	0.107	1.31 (0.99, 1.74)	0.056
	IL-5/ IFN-γ	28.51	22.94	0.99 (0.74, 1.33)	0.954	0.87 (0.67, 1.14)	0.324
	IL-13/ IFN-γ	13.2	5.96	0.89 (0.62, 1.27)	0.515	0.83 (0.59, 1.15)	0.253
SEA	IL-10/ IFN-γ	7.53	3.37	0.88 (0.63, 1.23)	0.444	0.88 (0.63, 1.22)	0.432
	IL-10/ IL-5	0.88	0.92	1.01 (0.82, 1.23)	0.955	1.05 (0.85, 1.28)	0.635
	IL-10/ IL-13	1.37	3.15	1.26 (0.89, 1.77)	0.175	1.31 (0.95, 1.83)	0.095

	IL-5/ IFN-γ	8.11	3.71	0.86 (0.59, 1.26)	0.435	0.81 (0.55, 1.16)	0.234
	IL-13/ IFN-γ	5.01	1.09	0.72 (0.50, 1.02)	0.064	0.71 (0.50, 1.00)	0.051
		Undetecta-ble asIgE n=83	Detectable asIgE[¶] n=320				
SWA	IL-10/ IFN-γ	**2.05**	**8.44**	**1.47 (1.07, 2.02)**	**0.019**	**1.46 (1.09, 1.94)**	**0.012**
	IL-10/ IL-5	0.22	0.26	0.99 (0.83, 1.20)	0.999	1.02 (0.86, 1.23)	0.749
	IL-10/ IL-13	0.36	0.72	1.09 (0.87, 1.37)	0.430	1.10 (0.88, 1.36)	0.370
	IL-5/ IFN-γ	**9.47**	**30.88**	**1.69 (1.21, 2.37)**	**0.003**	**1.58 (1.17, 2.14)**	**0.004**
	IL-13/ IFN-γ	5.62	11.42	1.31 (0.96, 1.77)	0.080	1.25 (0.96, 1.62)	0.093
SEA	IL-10/ IFN-γ	**2.97**	**7.79**	1.41 (0.99, 1.99)	0.055	**1.43 (1.03, 1.96)**	**0.032**
	IL-10/ IL-5	0.88	1.01	0.97 (0.73, 1.30)	0.878	0.99 (0.77, 1.28)	0.972
	IL-10/ IL-13	1.51	1.85	1.04 (0.76, 1.41)	0.785	1.05 (0.79, 1.39)	0.703
	IL-5/ IFN-γ	**3.37**	**7.38**	**1.58 (1.16, 2.15)**	**0.005**	**1.55 (1.15, 2.08)**	**0.005**
	IL-13/ IFN-γ	1.96	3.89	1.13 (0.92, 1.40)	0.233	1.14 (0.93, 1.39)	0.186

[†]All antibody concentrations in pg/ml.

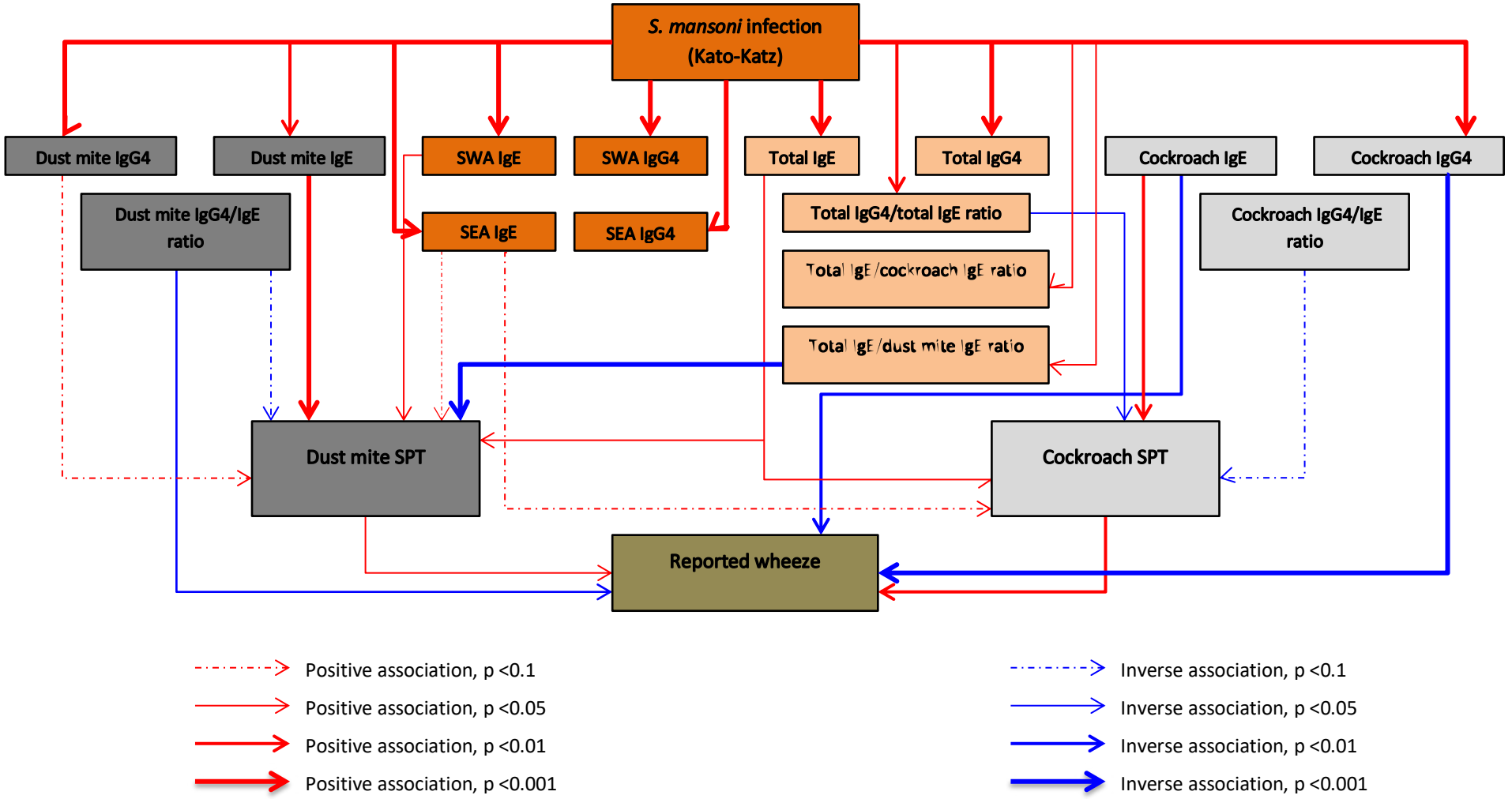
[‡]Geometric mean ratios and 95% confidence intervals adjusted for the survey design.

[§]SPT reactivity to any one of *Dermatophagoides* mix, Blomia tropicalis or *Blattella germanica.*

[¶]Detectable IgE to either *Dermatophagoides pteronyssinus* or *Blattella germanica*.

SmKK-: Kato-Katz negative result (*S. mansoni*), single stool sample; SmKK+: Kato-Katz positivity for *S. mansoni*, single stool sample; SWA: *Schistosoma* worm antigen; SEA: *Schistosoma* egg antigen; asIgE: allergen-specific IgE; GMR: geometric mean ratio; 95% CI: 95% confidence interval.

Figure S1. Summary of associations between antibody levels and i) *S. mansoni* infection, ii) SPT reactivity and iii) wheeze



Red and blue arrows denote positive and inverse associations, respectively. The thickness of the arrows shows the level of statistical significance. P values were obtained from linear regression analyses, after adjusting for survey design, age and sex. Furthermore, analyses assessing associations between antibody levels and SPT reactivity and wheeze were additionally adjusted for *S. mansoni* infection (Kato-Katz). SPT: skin prick test; SWA: *Schistosoma* worm antigen; SEA: *Schistosoma* egg antigen.

Table S2. Correlation between antibody profiles

	SWA IgE	SEA IgE	SWA IgG4	SEA IgG4	Dust mite IgE	Cockroach IgE	Dust mite IgG4	Cockroach IgG4	Total IgE	Total IgG4
SWA IgE	1									
SEA IgE	0.5202	1								
SWA IgG4	0.4409	0.2622	1							
SEA IgG4	0.3783	0.3074	0.6997	1						
Dust mite IgE	0.3823	0.2631	0.2462	0.218	1					
Cockroach IgE	0.2302	0.2895	0.0992	0.0707	0.3539	1				
Dust mite IgG4	0.1217	0.1702	0.2756	0.2425	0.1589	0.013	1			
Cockroach IgG4	0.1485	0.2062	0.2423	0.1701	0.1055	0.1014	0.5729	1		
Total IgE	0.5128	0.3095	0.4796	0.3764	0.3767	0.1575	0.1708	0.211	1	
Total IgG4	0.2201	0.2042	0.5052	0.4573	0.0861	0.0147	0.3902	0.3371	0.3977	1

Table shows Spearman's rank correlation coefficients (r_s), calculated to estimate strength of association between antibody responses. Hinkle and colleagues' criteria[1] were used to interpret r_s values: 0.7 – 1.00: high correlation; 0.5 – 0.7: moderate correlation; 0.3 – 0.5: low correlation; 0.00 – 0.3: little if any correlation.

Table S3. Associations between *S. mansoni* infection intensity and antibody and cytokine responses

A

Antigen	Cytokine	Geometric mean†				aGMR (95% CI) ‡		
		SmKK-	SmKK^{low}	SmKK^{mod}	SmKK^{heavy}	SmKK^{low}	SmKK^{mod}	SmKK^{heavy}
SWA	IFN-γ	1.16	2.67	**1.14**	0.45	1.49 (0.99, 2.25)	0.96 (0.70, 1.33)	**0.77 (0.61, 0.96)**
	IL-5	**14.92**	28.67	**80.23**	61.04	1.09 (0.82, 1.45)	**1.57 (1.09, 2.25)**	1.42 (0.95, 2.09)
	IL-13	7.01	15.58	17.63	19.92	1.11 (0.85, 1.45)	1.17 (0.85, 1.60)	1.17 (0.79, 1.72)
	IL-10	3.99	9.65	13.68	12.39	1.08 (0.79, 1.49)	1.18 (0.87, 1.61)	1.25 (1.03, 1.51)
SEA	IFN-γ	0.73	1.25	0.42	**0.29**	1.26 (0.96, 1.65)	0.92 (0.68, 1.25)	**0.78 (0.67, 0.92)**
	IL-5	5.02	6.18	3.08	**1.49**	1.10 (0.75, 1.62)	0.75 (0.49, 1.14)	**0.67 (0.48, 0.93)**
	IL-13	2.25	5.64	1.50	**0.76**	1.22 (0.98, 1.51)	0.78 (0.61, 1.02)	**0.66 (0.54, 0.83)**
	IL-10	3.19	7.32	4.63	2.45	1.12 (0.91, 1.38)	0.88 (0.67, 1.16)	0.78 (0.59, 1.05)

B

Antigen	Antibody/ antibody ratio	Geometric mean¶				aGMR (95% CI) ‡		
		SmKK-	SmKK^{low}	SmKK^{mod}	SmKK^{heavy}	SmKK^{low}	SmKK^{mod}	SmKK^{heavy}
SWA	IgE	**1080**	**2075**	**2544**	**2849**	**1.38 (1.08, 1.75)**	**1.48 (1.21, 1.82)**	**1.84 (1.52, 2.23)**
	IgG4	**4031**	**14132**	**26403**	**62356**	**2.69 (2.28, 3.16)**	**3.77 (2.82, 5.05)**	**5.75 (4.41, 7.50)**
SEA	IgE	**1412**	**1870**	**1953**	**1688**	**1.22 (1.06, 1.39)**	**1.50 (1.26, 1.78)**	**1.35 (1.06, 1.71)**
	IgG4	**18962**	**170274**	**229400**	**391440**	**4.45 (3.47, 5.71)**	**5.86 (4.66, 7.37)**	**7.04 (5.59, 8.87)**
Dust mite	IgE	**0.782**	3.926	**10.552**	**39.482**	1.05 (0.86, 1.27)	**1.33 (1.12, 1.56)**	**1.51 (1.14, 2.01)**
	IgG4	**0.001**	**0.059**	**0.274**	**5.939**	**1.50 (1.18, 1.91)**	**1.65 (1.31, 2.08)**	**2.51 (2.04, 3.07)**

	IgG4/IgE ratio	0.002	0.013	0.024	0.155	1.37 (0.62, 3.03)	1.10 (0.67, 1.81)	1.03 (0.37, 2.82)
Cockroach	IgE	18.87	15.60	16.66	28.37	0.97 (0.78, 1.21)	1.02 (0.74, 1.42)	1.02 (0.86, 1.19)
	IgG4	**0.002**	**0.091**	**0.147**	**3.349**	**1.43 (1.25, 1.65)**	**1.43 (1.22, 1.68)**	**2.01 (1.69, 2.39)**
	IgG4/IgE ratio	0.001	0.009	0.015	0.176	1.35 (0.91, 2.03)	1.08 (0.64, 1.83)	1.49 (0.91, 2.44)
	Total IgE	**969**	**2548**	**2208**	**4946**	**1.21 (1.01, 1.45)**	**1.19 (1.06, 1.35)**	**1.80 (1.62, 2.00)**
	Total IgG4	**51453**	**115588**	**235755**	**520703**	**1.57 (1.31, 1.89)**	**2.07 (1.53, 2.66)**	**2.42 (1.59, 3.66)**
	Total IgG4/ total IgE ratio	**52.16**	45	**107**	**100**	1.21 (0.96, 1.52)	**1.56 (1.17, 2.08)**	**1.39 (1.08, 1.79)**
	Total IgE/ cockroach IgE ratio	**3.79**	11.23	8.47	**19.30**	1.27 (0.92, 1.76)	1.08 (0.87, 1.36)	**1.64 (1.31, 2.04)**
	Total IgE/ dust mite IgE ratio	**0.562**	**1.66**	0.78	1.52	**1.17 (1.02, 1.32)**	1.04 (0.86, 1.26)	**1.17 (1.03, 1.34)**

†All cytokine concentrations in pg/ml.

¶All antibody concentrations in ng/ml.

‡Geometric mean ratios and 95% confidence intervals adjusted for the survey design, age and sex.

SmKK-: Kato-Katz negative result (*S. mansoni*), single stool sample; **SmKK^{low}**: Kato-Katz positivity, low infection intensity (1-99 eggs/g); **SmKK^{mod}**: Kato-Katz positivity, moderate infection intensity (100-399 eggs/g); **SmKK^{heavy}**: Kato-Katz positivity, heavy infection intensity (≥400 eggs/g); **SWA**: *Schistosoma* worm antigen; **SEA**: *Schistosoma* egg antigen; **aGMR**: adjusted geometric mean ratio; **95% CI**: 95% confidence interval.

Table S4. Comparison of cytokine and antibody responses between SmKK-CAA+ participants and (i) SmKK-CCA- and (ii) SmKK+CAA+/- individuals[†]

A

Antigen	Cytokine	Geometric mean			aGMR (95% CI) [‡§]	
		SmKK-CCA+	*SmKK-CCA-*	*SmKK+CCA+/-*	*SmKK-CCA-*	*SmKK+CCA+/-*
SWA	IFN-γ	1.83	**0.57**	1.33	**0.71 (0.52, 0.97)**	0.90 (0.66, 1.23)
	IL-5	17.81	10.80	40.28	0.80 (0.47, 1.35)	1.38 (0.95, 2.01)
	IL-13	9.54	5.91	14.62	0.71 (0.43, 1.19)	1.06 (0.68, 1.65)
	IL-10	5.72	**2.15**	15.27	**0.68 (0.55, 0.86)**	1.25 (0.93, 1.69)
SEA	IFN-γ	0.57	1.09	0.46	0.98 (0.56, 1.74)	0.96 (0.63, 1.46)
	IL-5	15.15	**2.35**	3.26	**0.49 (0.33, 0.74)**	0.69 (0.46, 1.04)
	IL-13	2.79	**1.15**	1.85	**0.66 (0.44, 1.00)**	0.96 (0.56, 1.65)
	IL-10	5.40	**1.63**	5.45	**0.66 (0.45, 0.98)**	0.97 (0.66, 1.41)

B

Antigen	Antibody / antibody ratio	Geometric mean			aGMR (95% CI) [‡]	
		SmKK-CCA+	*SmKK-CCA-*	*SmKK+CCA+/-*	*SmKK-CCA-*	*SmKK+CCA+/-*
SWA	IgE	1244	843	**3058**	0.92 (0.50, 1.67)	**1.67 (1.13, 2.48)**
	IgG4	4830	**2291**	**27587**	**0.60 (0.45, 0.79)**	**2.85 (2.01, 4.03)**
SEA	IgE	1473	1453	**1927**	0.76 (0.43, 1.37)	**1.42 (1.14, 1.76)**
	IgG4	33789	**6311**	**218820**	**0.30 (0.14, 0.65)**	**3.63 (2.28, 5.77)**
Dust mite	IgE	0.61	0.45	**22.59**	1.06 (0.82, 1.38)	**1.46 (1.16, 1.84)**

	IgG4	0.001	0.001	**0.221**	1.03 (0.75, 1.39)	**1.46 (1.12, 1.92)**
	IgG4/IgE ratio	0.004	0.004	0.015	0.79 (0.32, 1.95)	0.57 (0.27, 1.21)
Cockroach	IgE	20.17	9.61	17.85	0.92 (0.74, 1.14)	0.98 (0.75, 1.27)
	IgG4	0.079	0.005	**0.609**	0.82 (0.63, 1.06)	**1.26 (1.09, 1.47)**
	IgG4/IgE ratio	0.004	0.000	0.035	0.86 (0.49, 1.51)	1.24 (0.78, 1.96)
	Total IgE	696	1222	**3302**	1.16 (0.82, 1.65)	**1.69 (1.15, 2.48)**
	Total IgG4	44075	5543	**282711**	0.91 (0.67, 1.21)	**1.79 (1.10, 2.92)**
	Total IgG4/ total IgE ratio	63.31	43.75	80.71	0.83 (0.65, 1.05)	1.04 (0.65, 1.65)
	Total IgE/ cockroach IgE ratio	2.26	5.65	19.62	1.04 (0.76, 1.41)	1.40 (0.90, 2.18)
	Total IgE/ dust mite IgE ratio	0.29	0.82	1.33	1.03 (0.90, 1.18)	1.14 (0.95, 1.35)

[†]279 (cytokine responses), 1242 (allergen and *S. mansoni*-specific antibody responses) and 319 (total antibody responses) individuals missing CCA result; not included in analysis

[‡]Geometric mean ratios and 95% confidence intervals adjusted for the survey design, age and sex.

[§]SmKK-CCA+ used as the base category

SmKK-: Kato-Katz negative result (*S. mansoni*), single stool sample; **SmKK+:** Kato-Katz positive result (*S. mansoni*), single stool sample; **CCA-:** Negative result for *S. mansoni* circulating cathodic antigen; **CCA+:** Positive result for *S. mansoni* circulating cathodic antigen; **SmKK+CCA+/-:** SmKK+ result, irrespective of CCA result; **SWA**: *Schistosoma* worm antigen; **SEA**: *Schistosoma* egg antigen; **aGMR**: adjusted geometric mean ratio; **95% CI**: 95% confidence interval.

References

1. Hinkle DE, Wiersma W, Jurs SG. Applied Statistics for the Behavioral Sciences 5th Edition: Boston: Houghton Mifflin; 2003.

5.4 Chapter 5: extra, unpublished results

5.4.1 LaVIISWA year three outcome survey: impact of intensive versus standard anthelminthic treatment on IgE and IgG4 profiles

The results reported in this chapter, so far, are from the LAVIISWA baseline survey, which preceded three years of community-based intensive versus standard anthelminthic treatment. Allergy-related trial outcomes were assessed after the three years of intervention, in a household-based survey: for all primary outcomes, there was no evidence of a difference in prevalence between intensive and standard trial arms (**Research Paper 7, Appendix 3,** this thesis). The present analysis also shows no evidence of an impact of intensive versus standard anthelminthic treatment on IgE and IgG4 profiles (**Table E1**, below).

Table E1. Impact of community-based intensive versus standard anthelminthic treatment on IgE and IgG4 profiles in the LaVIISWA outcome survey

Outcome	Geometric mean		Unadjusted		Adjusted for age & sex	
	Standard	**Intensive**	**GMR (95% CI)**	**p**	**GMR (95% CI)**	**p**
***Schistosoma*-specific antibody concentration (ELISA, ng/ml)**						
SWA-specific IgE	4457	4451	0.99 (0.88, 1.14)	0.98	0.99 (0.87, 1.14)	0.95
SWA- specific IgG4	91628	73379	0.79 (0.50, 1.23)	0.28	0.78 (0.52, 1.17)	0.22
SEA- specific IgE	4196	4494	1.07 (0.90, 1.26)	0.45	1.06 (0.90, 1.25)	0.44
SEA- specific IgG4	112276	73352	0.64 (0.29, 1.41)	0.25	0.65 (0.29, 1.45)	0.28
asIgE concentration (ImmunoCAP, kU/L)						
House dust mite	0.158	0.129	0.78 (0.51, 1.17)	0.22	0.76 (0.51, 1.13)	0.17
Cockroach	0.342	0.289	0.82 (0.55, 1.22)	0.31	0.81 (0.55, 1.20)	0.28
Peanut	0.074	0.066	0.89 (0.64, 1.23)	0.47	0.89 (0.65, 1.23)	0.49
asIgE and IgG4 concentration (ELISA, ng/ml)						
House dust mite-specific IgE	26.5	23.0	0.82 (0.48, 1.39)	0.45	0.81 (0.49, 1.35)	0.40
House dust mite-specific IgG4	13.7	12.2	0.86 (0.63, 1.17)	0.32	0.89 (0.68, 1.2)	0.37
Cockroach-specific IgE	32.9	39.7	1.20 (0.76, 1.89)	0.41	1.28 (0.81, 2.01)	0.28
Cockroach-specific IgG4	14.6	13.4	0.91 (0.57, 1.47)	0.69	0.93 (0.59, 1.46)	0.75
Total IgE (ImmunoCAP, kU/L)	754	618	0.80 (0.61, 1.04)	0.09	0.79 (0.62, 1.00)	0.05

Total IgG4 (ELISA, ng/ml)	22170	17515	0.78 (0.55, 1.10)	0.15	0.78 (0.55, 1.08)	0.13
Total IgG4/ total IgE ratio (ELISA/ImmunoCAP)	36.5	38.7	1.07 (0.88, 1.30)	0.50	1.05 (0.87, 1.26)	0.60
Total IgE/ cockroach IgE ratio (ImmunoCAP)	2081	1971	0.94 (0.71, 1.25)	0.66	0.94 (0.71, 1.25)	0.65
Total IgE/ dust mite IgE ratio (ImmunoCAP)	3967	3929	0.99 (0.71, 1.39)	0.97	0.98 (0.69, 1.38)	0.92
Total IgE/ peanut IgE ratio (ImmunoCAP)	10581	9717	0.90 (0.69, 1.18)	0.43	0.90 (0.68, 1.19)	0.44

asIgE: allergen-specific IgE; **GMR:** geometric mean ratio; **CI:** confidence interval

5.4.2 LaVIISWA year three outcome survey: relationships between antibody responses, KK positivity and allergy

Table E2 (below) shows associations between antibody responses and *S. mansoni* KK positivity and allergy in the outcome survey (both trial arms, combined). Kato-Katz positivity was strongly positively associated with SWA- and SEA-specific IgE and IgG4 and with total IgE, total IgG4, total IgG4 / total IgE ratios and total IgE / asIgE ratios. Total and allergen-specific IgE were positively associated with cockroach and HDM SPT reactivity. In contrast, cockroach SPT was inversely associated with total IgE / allergen-specific IgE ratios, and HDM SPT reactivity was inversely associated with SWA-specific IgE, total IgE / allergen-specific IgE ratios, and HDM IgG4 / IgE ratios.

These results are similar to those observed at baseline (Research paper 2, above). The main difference was in the association between antibodies and wheeze: positive associations with SWA-specific IgG4 and HDM-specific IgE were observed in the outcome survey (**Table E2**) unlike in the baseline survey, where any associations, when significant, were inverse. Associations with wheeze should be interpreted with caution, as prevalence of wheeze was very low (Research Paper 1).

Table E2. LaVIISWA year three outcome survey: associations between antibody (IgE and IgG4) levels and Kato-Katz positivity (*S. mansoni*), SPT reactivity and reported wheeze

Antigen	Antibody / antibody ratio	Geometric mean	aGMR (95% CI) ‡#	p value

		SmKK-	*SmKK+*		
SWA	IgE§	**4009**	**5901**	**1.43 (1.26, 1.62)**	**<0.001**
	IgG4§	**54728**	**201397**	**3.33 (2.00, 5.54)**	**<0.001**
SEA	IgE§	**4026**	**5328**	**1.36 (1.13, 1.64)**	**0.002**
	IgG4§	**49572**	**371634**	**5.33 (2.77, 10.23)**	**<0.001**
Dust mite (ImmunoCAP)	IgE¶	0.133	0.209	1.50 (0.93, 2.42)	0.090
Dust mite (ELISA)	IgE§	23.46	33.42	1.33 (0.84, 2.12)	0.214
	IgG4§	12.28	14.18	1.11 (0.82, 1.50)	0.480
	IgG4/IgE ratio§	12.03	9.89	0.93 (0.61, 1.41)	0.711
Cockroach (ImmunoCAP)	IgE¶	0.293	0.407	1.28 (0.88, 1.89)	0.188
Cockroach (ELISA)	IgE§	31.53	41.93	1.13 (0.56, 2.28)	0.731
	IgG4§	11.89	20.01	1.52 (0.87, 2.67)	0.135
	IgG4/IgE ratio§	**5.06**	**6.92**	**1.52 (1.07, 2.15)**	**0.021**
Peanut (ImmunoCAP)	IgE¶	0.062	0.095	1.28 (0.96, 1.69)	0.089
	Total IgE¶	**536.7**	**1305.7**	**2.26 (1.78, 2.87)**	**<0.001**
	Total IgG4§	**17026**	**30653**	**1.98 (1.22, 3.23)**	**0.008**
	Total IgG4/ total IgE ratio	**41.36**	**28.62**	**0.77 (0.64, 0.94)**	**0.012**
	Total IgE/ cockroach IgE ratio¶	**1662.6**	**3138.3**	**1.96 (1.38, 2.77)**	**0.001**
	Total IgE/ dust mite IgE ratio¶	**3143.31**	**5818.10**	**1.86 (1.13, 3.08)**	**0.017**
	Total IgE/ peanut IgE ratio¶	**8968.01**	**14121.30**	**1.74 (1.32, 2.30)**	**<0.001**

		Cockroach SPT-	*Cockroach SPT+*		
SWA	IgE§	4481	4316	1.04 (0.85, 1.29)	0.685
	IgG4§	86035	58160	0.87 (0.36, 2.13)	0.754
SEA	IgE§	4348	4327	1.00 (0.86, 1.15)	0.958
	IgG4§	90368	93691	1.23 (0.52, 2.92)	0.622
Dust mite (ImmunoCAP)	IgE¶	**0.116**	**0.607**	**5.67 (3.43, 9.38)**	**<0.001**
Dust mite (ELISA)	IgE§	25.03	23.23	0.89 (0.34, 2.36)	0.812
	IgG4§	13.05	11.86	1.14 (0.73, 1.78)	0.541
	IgG4/IgE ratio§	11.61	12.98	1.58 (0.82, 3.04)	0.162
Cockroach (ImmunoCAP)	IgE¶	**0.241**	**1.982**	**8.25 (5.16, 13.19)**	**<0.001**
Cockroach (ELISA)	IgE§	32.87	73.13	1.32 (0.46, 3.83)	0.592
	IgG4§	13.31	19.16	1.25 (0.64, 2.44)	0.507

	IgG4/IgE ratio§	5.41	4.79	1.28 (0.66, 2.47)	0.448
Peanut (ImmunoCAP)	IgE¶	**0.06**	**0.12**	**2.32 (1.57, 3.44)**	**<0.001**
	Total IgE¶	**652.99**	**934.25**	**1.71 (1.21, 2.44)**	**0.004**
	Total IgG4§	18805	27184	1.29 (0.77, 2.16)	0.317
	Total IgG4/ total IgE ratio	38.17	32.95	0.71 (0.50, 1.02)	0.060
	Total IgE/ cockroach IgE ratio¶	**2516**	**474**	**0.23 (0.16, 0.34)**	**<0.001**
	Total IgE/ dust mite IgE ratio¶	**4606**	**1421**	**0.36 (0.28, 0.46)**	**<0.001**
	Total IgE/ peanut IgE ratio¶	**10551**	**7679**	**0.73 (0.57, 0.92)**	**0.009**

		Dust mite SPT-	*Dust mite SPT+*		
SWA	IgE§	4520	3969	0.95 (0.83, 1.08)	0.385
	IgG4§	**90655**	**36677**	**0.49 (0.27, 0.87)**	**0.017**
SEA	IgE§	4389	4005	0.96 (0.83, 1.10)	0.524
	IgG4§	89975	96456	1.39 (0.73, 2.62)	0.303
Dust mite (ImmunoCAP)	IgE¶	**0.10**	**2.27**	**31.0 (17.66, 54.36)**	**<0.001**
Dust mite (ELISA)	IgE§	**20.14**	**123.69**	**8.45 (3.54, 20.18)**	**<0.001**
	IgG4§	12.99	12.39	1.26 (0.65, 2.43)	0.481
	IgG4/IgE ratio§	**13.40**	**4.44**	**0.31 (0.18, 0.54)**	**<0.001**
Cockroach (ImmunoCAP)	IgE¶	**0.28**	**0.91**	**3.50 (2.09, 5.87)**	**<0.001**
Cockroach (ELISA)	IgE§	35.46	41.90	1.25 (0.53, 2.94)	0.604
	IgG4§	14.53	10.42	0.85 (0.32, 2.20)	0.720
	IgG4/IgE ratio§	5.45	4.87	0.95 (0.39, 2.25)	0.898
Peanut (ImmunoCAP)	IgE¶	**0.07**	**0.09**	**1.77 (1.16, 2.72)**	**0.011**
	Total IgE¶	675.19	744.28	1.25 (0.93, 1.69)	0.137
	Total IgG4§	20462	14579	0.55 (0.27, 1.12)	0.095
	Total IgG4/ total IgE ratio	38.14	33.31	0.71 (0.44, 1.16)	0.162
	Total IgE/ cockroach IgE ratio¶	**2289**	**778**	**0.38 (0.28, 0.51)**	**<0.001**
	Total IgE/ dust mite IgE ratio¶	**5534**	**335**	**0.05 (0.04, 0.08)**	**<0.001**

	Total IgE/ peanut IgE ratio¶	10394	8315	0.69 (0.48, 1.01)	0.054

		No wheeze	*Wheeze*		
SWA	IgE§	4424	5038	1.09 (0.86, 1.38)	0.448
	IgG4§	**79765**	**157428**	**2.06 (1.14, 3.72)**	**0.019**
SEA	IgE§	4346	3899	0.97 (0.79, 1.19)	0.769
	IgG4§	90025	134327	1.14 (0.37, 3.48)	0.815
Dust mite (ImmunoCAP)	IgE¶	**0.139**	**0.343**	**3.83 (1.08, 13.61)**	**0.039**
Dust mite (ELISA)	IgE§	**24.15**	**68.35**	**6.64 (1.06, 41.64)**	**0.044**
	IgG4§	12.7	27.07	1.43 (0.37, 5.44)	0.590
	IgG4/IgE ratio§	11.94	11.43	0.59 (0.13, 2.62)	0.476
Cockroach (ImmunoCAP)	IgE¶	0.312	0.47	2.12 (0.67, 6.69)	0.192
Cockroach (ELISA)	IgE§	34.59	87.54	5.57 (0.94, 32.96)	0.058
	IgG4§	13.53	41.40	2.33 (0.92, 5.88)	0.073
	IgG4/IgE ratio§	5.33	7.72	0.89 (0.20, 3.86)	0.867
Peanut (ImmunoCAP)	IgE¶	0.069	0.088	1.26 (0.81, 1.96)	0.296
	Total IgE¶	667.95	1199.8	1.62 (0.95, 2.78)	0.076
	Total IgG4§	19456	27638	1.33 (0.76, 2.33)	0.311
	Total IgG4/ total IgE ratio	38.12	29.50	0.81 (0.45, 1.47)	0.471
	Total IgE/ cockroach IgE ratio¶	1990.86	2570.97	0.84 (0.36, 1.97)	0.675
	Total IgE/ dust mite IgE ratio¶	3968.74	3160.90	0.49 (0.17, 1.44)	0182
	Total IgE/ peanut IgE ratio¶	9987.71	14279.90	1.29 (0.79, 2.11)	0.300

§Antibody levels detected by ELISA. Concentrations are in ng/ml.

¶Antibody levels detected by ImmunoCAP. Concentrations are in kU/L.

‡All geometric mean ratios and 95% confidence intervals adjusted for survey design, age and sex.

#Geometric mean ratios and 95% confidence intervals for associations between antibody levels and SPT reactivity and wheeze were additionally adjusted for SmKK result.

SWA: *Schistosoma* worm antigen; **SEA:** *Schistosoma* egg antigen; **SmKK-**: Kato-Katz negative result (*S. mansoni*), single stool sample; **SmKK^{+}**: Kato-Katz positive result, single stool sample; **aGMR:** adjusted geometric mean ratio; **95% CI:** 95% confidence interval.

5.4.3 Urban survey of allergy-related outcomes: relationships between antibody responses, S. mansoni Kato-Katz/PCR positivity and allergy

In the urban survey, total, SWA-, SEA- and allergen-specific IgE and IgG4 concentrations were all positively associated with *S. mansoni* infection (**Table E3**, below), mirroring observations in the rural baseline and outcome surveys (presented above). Total and allergen-specific IgE and IgG4 concentrations were positively associated with cockroach and HDM SPT reactivity, while allergen-specific IgG4/ IgE ratios, total IgG4/ total IgE ratios and total IgE/ allergen-specific IgE ratios were inversely associated with the same outcomes.

HDM- and SWA-specific IgG4 were positively associated with wheeze, while SWA-specific IgE was inversely associated with the same outcome. Similar to the rural baseline survey, prevalence of wheeze was very low (shown in Research Paper 1), hence associations with wheeze should be interpreted with caution.

Table E3. Urban survey: associations between antibody (IgE and IgG4) levels and Kato-Katz / PCR positivity (*S. mansoni*), SPT reactivity and reported wheeze

Antigen	Antibody / antibody ratio	Geometric mean		aGMR (95% CI) ‡#	p value
		Sm-	*Sm+*		
SWA	IgE§	**2188**	**3949**	**1.75 (1.58, 1.94)**	**<0.001**
	IgG4§	**12134**	**80715**	**5.93 (3.76, 9.38)**	**<0.001**
SEA	IgE§	**2434**	**4660**	**1.88 (1.63, 2.16)**	**<0.001**
	IgG4§	**981**	**83098**	**65.9 (41.4, 104.8)**	**<0.001**
Dust mite (ImmunoCAP)	IgE¶	0.18	0.25	1.39 (0.65, 3.00)	0.379
Dust mite (ELISA)	IgE§	**16.57**	**38.20**	**2.08 (1.05, 4.12)**	**0.037**
	IgG4§	19.56	29.02	1.62 (0.99, 2.66)	0.056
	IgG4/IgE ratio§	20.90	17.53	0.95 (0.56, 1.61)	0.839
Cockroach (ImmunoCAP)	IgE¶	0.18	0.22	1.09 (0.63, 1.88)	0.745
Cockroach (ELISA)	IgE§	**18.04**	**38.12**	**1.91 (1.03, 3.53)**	**0.040**
	IgG4§	**8.78**	**24.28**	**2.54 (1.37, 4.71)**	**0.005**
	IgG4/IgE ratio§	5.61	5.53	0.99 (0.62, 1.62)	0.994
Peanut (ImmunoCAP)	IgE¶	**0.03**	**0.06**	**2.06 (1.23, 3.46)**	**<0.008**

	Total IgE¶	**143**	**360**	**2.62 (1.81, 3.79)**	**<0.001**
	Total IgG4§	**12197**	**25104**	**1.96 (1.45, 2.64)**	**<0.001**
	Total IgG4/ total IgE ratio	99.57	84.34	0.73 (0.38, 1.41)	0.327
	Total IgE/ cockroach IgE ratio¶	**807**	**1674**	**2.39 (1.56, 3.66)**	**<0.001**
	Total IgE/ dust mite IgE ratio¶	837	1489	1.86 (0.95, 3.64)	0.068
	Total IgE/ peanut IgE ratio¶	4788	6482	1.38 (0.96, 1.98)	0.076

		Cockroach SPT-	*Cockroach SPT+*		
SWA	IgE§	2367	2551	1.08 (0.99, 1.16)	0.057
	IgG4§	15174	21505	1.41 (0.87, 2.30)	0.152
SEA	IgE§	**2645**	**3187**	**1.13 (1.03, 1.23)**	**0.012**
	IgG4§	**2131**	**1171**	**0.28 (0.12, 0.63)**	**0.004**
Dust mite (ImmunoCAP)	IgE¶	**0.12**	**4.74**	**40.19 (19.2, 84.24)**	**<0.001**
Dust mite (ELISA)	IgE§	**12**	**257**	**20.92 (11.9, 36.66)**	**<0.001**
	IgG4§	**20.9**	**27.4**	**1.61 (1.25, 2.09)**	**0.001**
	IgG4/IgE ratio§	**28.01**	**4.38**	**0.18 (0.13, 0.23)**	**<0.001**
Cockroach (ImmunoCAP)	IgE¶	**0.13**	**1.70**	**13.49 (7.19, 25.32)**	**<0.001**
Cockroach (ELISA)	IgE§	**16.39**	**79.46**	**4.64 (2.00, 10.78)**	**0.001**
	IgG4§	**9.08**	**23.59**	**3.45 (1.76, 6.77)**	**0.001**
	IgG4/IgE ratio§	5.88	4.21	0.81 (0.42, 1.56)	0.514
Peanut (ImmunoCAP)	IgE¶	**0.03**	**0.13**	**7.37 (2.58, 21.09)**	**0.001**
	Total IgE¶	**141**	**505**	**4.19 (2.80, 6.28)**	**<0.001**
	Total IgG4§	12517	17417	1.32 (0.83, 2.12)	0.223
	Total IgG4/ total IgE ratio	**109.80**	**50.05**	**0.41 (0.24, 0.70)**	**0.002**
	Total IgE/ cockroach IgE ratio¶	**1071**	**302**	**0.31 (0.15, 0.64)**	**0.003**
	Total IgE/ dust mite IgE ratio¶	**1227**	**110**	**0.10 (0.04, 0.25)**	**<0.001**
	Total IgE/ peanut IgE ratio¶	5231	4002	0.62 (0.26, 1.47)	0.261

		Dust mite SPT-	*Dust mite SPT+*		
SWA	IgE§	2365	2526	0.99 (0.91, 1.09)	0.915
	IgG4§	15601	19133	0.88 (0.44, 1.75)	0.707
SEA	IgE§	2655	3018	1.02 (0.86, 1.19)	0.835
	IgG4§	1884	2337	0.55 (0.26, 1.17)	0.113
Dust mite (ImmunoCAP)	IgE¶	**0.09**	**4.86**	**47.48 (24.1, 93.68)**	**<0.001**
Dust mite (ELISA)	IgE§	**10**	**298**	**24.69 (12.9, 47.39)**	**<0.001**
	IgG4§	**20.05**	**32.33**	**2.10 (1.53, 2.87)**	**<0.001**
	IgG4/IgE ratio§	**30.46**	**4.17**	**0.17 (0.11, 0.28)**	**<0.001**
Cockroach (ImmunoCAP)	IgE¶	**0.14**	**0.70**	**3.99 (1.84, 8.67)**	**0.001**
Cockroach (ELISA)	IgE§	18.04	39.09	2.17 (0.89, 5.26)	0.085
	IgG4§	**9.25**	**17.54**	**1.92 (1.17, 3.15)**	**0.012**
	IgG4/IgE ratio§	5.40	6.37	1.22 (0.65, 2.30)	0.526
Peanut (ImmunoCAP)	IgE¶	**0.03**	**0.08**	**3.58 (1.60, 7.99)**	**0.003**
	Total IgE¶	**142**	**347**	**2.46 (1.65, 3.67)**	**<0.001**
	Total IgG4§	12527	16210	1.26 (0.85, 1.87)	0.233
	Total IgG4/ total IgE ratio	106.99	70.48	0.65 (0.40, 1.05)	0.073
	Total IgE/ cockroach IgE ratio¶	1024	506	0.61 (0.35, 1.07)	0.080
	Total IgE/ dust mite IgE ratio¶	**1530**	**75**	**0.05 (0.03, 0.10)**	**<0.001**
	Total IgE/ peanut IgE ratio¶	5238	4212	0.71 (0.39, 1.29)	0.244

		No wheeze	*Wheeze*		
SWA	IgE§	**2386**	**2344**	**0.87 (0.76, 0.99)**	**0.033**
	IgG4§	**15235**	**43668**	**2.21 (1.22, 3.99)**	**0.011**
SEA	IgE§	2716	2842	0.84 (0.68, 1.03)	0.087
	IgG4§	1798	12209	1.98 (0.29, 13.37)	0.467
Dust mite (ImmunoCAP)	IgE¶	0.18	0.75	2.18 (0.44, 10.89)	0.325
Dust mite (ELISA)	IgE§	18.51	25.09	0.72 (0.08, 6.66)	0.760
	IgG4§	**21.83**	**50.53**	**3.21 (1.09, 9.39)**	**0.035**
	IgG4/IgE ratio§	21.52	40.46	4.17 (0.63, 27.42)	0.130
Cockroach (ImmunoCAP)	IgE¶	0.18	0.39	1.30 (0.24, 6.89)	0.746

Cockroach (ELISA)	IgE§	21.24	11.49	0.42 (0.08, 2.24)	0.295
	IgG4§	10.45	7.87	0.80 (0.19, 3.22)	0.744
	IgG4/IgE ratio§	5.58	5.07	1.35 (0.41, 4.43)	0.608
Peanut (ImmunoCAP)	IgE¶	0.03	0.09	2.45 (0.37, 16.21)	0.334
	Total IgE¶	156	354	1.96 (0.73, 5.23)	0.170
	Total IgG4§	13545	16560	0.97 (0.19, 4.84)	0.968
	Total IgG4/ total IgE ratio	112.00	55.98	0.45 (0.06, 3.32)	0.411
	Total IgE/ cockroach IgE ratio¶	863	918	1.49 (0.70, 3.20)	0.284
	Total IgE/ dust mite IgE ratio¶	863	497	0.94 (0.19, 4.71)	0.938
	Total IgE/ peanut IgE ratio¶	4814	3829	0.85 (0.32, 2.22)	0.721

§Antibody levels detected by ELISA. Concentrations are in ng/ml.

¶Antibody levels detected by ImmunoCAP. Concentrations are in kU/L.

‡All geometric mean ratios and 95% confidence intervals adjusted for survey design, age and sex.

#Geometric mean ratios and 95% confidence intervals for associations between antibody levels and SPT reactivity and wheeze were additionally adjusted for Sm result.

SWA: *Schistosoma* worm antigen; **SEA:** *Schistosoma* egg antigen; **Sm-**: Kato-Katz/PCR negative result (*S. mansoni*), single stool sample; **Sm$^+$**: Kato-Katz/PCR positive result, single stool sample; **aGMR:** adjusted geometric mean ratio; **95% CI:** 95% confidence interval.

5.4.4 Urban-rural differences in IgE and IgG4 profiles

Concentrations of total, SWA-, SEA- and allergen-specific IgE and IgG4 differed between the urban and rural (LaVIISWA outcome) survey (**Table E4**, below). Concentrations of total, SWA- and SEA-specific antibodies were higher in the rural survey. This may be attributed to the higher prevalence of *S. mansoni* infection in rural compared to the urban survey. Crude cockroach and peanut extract-specific IgE levels were also higher in the rural survey. Crude allergen extracts may contain cross-reactive components that are also expressed by helminths (which are more prevalent in the rural setting). Unexpectedly, HDM-specific IgG4, HDM IgG4/ IgE and total IgG4/ total IgE ratios were higher in the urban survey, and HDM-specific IgE levels were similar between the two settings.

Table E4. Urban-rural comparisons of IgE and IgG4 profiles

	Urban survey Median (IQR)	Rural survey Median (IQR)	p value
***Schistosoma*-specific antibodies**			
SWA-specific IgE*	2229.4 (1543.4, 3738.2)	**4962.1 (3006.6, 6727.7)**	<0.001
SWA-specific IgG4*	42718 (24995.3, 71493.8)	**108848.3 (51932.6, 273563.7)**	<0.001
SEA-specific IgE*	2707.1 (1802.2, 4517.3)	**4589.7 (3063.3, 6581.8)**	<0.001
SEA-specific IgG4*	30886.5 (0.0, 101818.1)	**274995.7 (70015.25, 839375.0)**	<0.001
Allergen-specific antibodies / antibody ratios			
House dust mite (HDM)			
IgE**	0.1 (0.0, 0.7)	0.2 (0.0, 0.6)	0.141
IgE*	0.1 (0.1, 965.2)	0.1 (0.1, 1050.4)	0.211
IgG4*	**15.6 (3.2, 100.2)**	8.4 (2.1, 47.0)	<0.001
IgG4/IgE ratio*	**8.3 (0.0, 319.3)**	1.0 (0.0, 99.4)	0.002
Cockroach			
IgE**	0.1 (0.0, 0.6)	**0.4 (0.1, 1.5)**	0.006
IgE*	0.1 (0.1, 816.9)	**75.3 (0.1, 1086.2)**	0.006
IgG4*	0.1 (0.1, 130.8)	**0.1 (0.1, 186.8)**	0.017
IgG4/IgE ratio*	1.0 (0.1, 1.0)	1.0 (0.0, 1.0)	0.973
Peanut-specific IgE**	0.0 (0.0, 0.1)	**0.1 (0.0, 0.2)**	<0.001
Total antibodies / antibody ratios			
Total IgE**	158.8 (56.7, 522.5)	**672.2 (249.5, 1942.5)**	<0.001
Total IgG4*	27051.2 (14942.4, 37539.6)	**32907.6 (19501.6, 47302)**	0.001
Total IgG4* / total IgE ratio**	**107.2 (377.0, 38.9)**	36.3 (12.9, 104.3)	<0.001
Total IgE / cockroach IgE ratio**	938.8 (330.6, 2472.8)	**2286.3 (683.9, 7124.1)**	<0.001
Total IgE / dust mite IgE ratio**	1371.3 (361.4, 3195.4)	**4938.6 (1572.5, 12029.1)**	<0.001
Total IgE / peanut IgE ratio**	6262.3 (2138.0, 15152.3)	**14243.1 (5827.2, 25282.8)**	<0.001

P values were obtained from survey design-based linear regression. Adjusting for age and sex differences had no significant impact on observed differences.

*Antibody levels detected by ELISA. Concentrations are in ng/ml.

**Antibody levels detected by ImmunoCAP. Concentrations are in kU/L.

IQR: Interquartile range; **SEA:** Schistosoma egg antigen; **SWA:** Schistosoma adult worm antigen

5.4.5 Associations between antibody responses and asthma status among schoolchildren

The asthma case-control study (described in the methods chapter) provided a unique opportunity to assess associations between allergy-related disease (asthma) and total, schistosome- and allergen-specific IgE and IgG4 concentrations. Total and allergen-specific IgE, but not IgG4 levels were strongly positively associated with asthma (**Table E5**, below). The perception that high IgG4 / IgE ratios and total / allergen-specific IgE ratios may be protective against clinical allergy is supported by analyses in the urban survey and the rural surveys above. Findings from the asthma case-control study provide further support: asthmatic schoolchildren had lower allergen-specific IgG4 / IgE ratios and total IgE / allergen-specific IgE ratios compared to non-asthmatic controls.

Table E5. Asthma case-control study: associations between antibody (IgE and IgG4) levels and asthma status

Antigen	Antibody / antibody ratio	Geometric mean		aGMR (95% CI) ‡	p value
		Non-asthmatic controls	*Asthmatics*		
SWA	IgE§	1694	1957	1.14 (0.88, 1.47)	0.312
	IgG4§	13247	14775	1.12 (0.57, 2.20)	0.743
SEA	IgE§	2051	2531	1.19 (0.90, 1.57)	0.233
	IgG4§	2351	1467	0.61 (0.21, 1.80)	0.372
Dust mite (ImmunoCAP)	IgE¶	**0.33**	**1.56**	**4.79 (2.72, 8.44)**	**<0.001**
Dust mite (ELISA)	IgE§	**20.32**	**99.39**	**4.99 (2.33, 10.72)**	**<0.001**
	IgG4§	72.05	58.37	0.89 (0.55, 1.44)	0.625
	IgG4/IgE ratio§	**51.52**	**12.23**	**0.24 (0.12, 0.48)**	**<0.001**
Cockroach (ImmunoCAP)	IgE¶	**0.32**	**0.64**	**2.03 (1.39, 2.97)**	**<0.001**
Cockroach (ELISA)	IgE§	**19.76**	**51.35**	**2.65 (1.31, 5.34)**	**0.007**
	IgG4§	20.22	22.13	1.11 (0.61, 2.0)	0.736
	IgG4/IgE ratio§	8.81	5.35	0.62 (0.33, 1.16)	0.133
Peanut (ImmunoCAP)	IgE¶	0.06	0.08	1.37 (0.97, 1.92)	0.071
	Total IgE¶	**252**	**372**	**1.53 (1.13, 2.05)**	**0.005**
	Total IgG4§	13549	16837	1.22 (0.69, 2.15)	0.486

Total IgG4/ total IgE ratio	82.85	62.37	0.72 (0.48, 1.07)	0.106
Total IgE/ cockroach IgE ratio¶	**789.08**	**590.89**	**0.75 (0.57, 0.99)**	**0.047**
Total IgE/ dust mite IgE ratio¶	**779**	**249**	**0.33 (0.21, 0.50**	**<0.001**
Total IgE/ peanut IgE ratio¶	4500	4840	1.08 (0.81, 1.42)	0.605

§Antibody levels detected by ELISA. Concentrations are in ng/ml.

¶Antibody levels detected by ImmunoCAP. Concentrations are in kU/L.

‡All geometric mean ratios and 95% confidence intervals adjusted for age and sex.

SWA: *Schistosoma* worm antigen; **SEA:** *Schistosoma* egg antigen; **aGMR:** adjusted geometric mean ratio; **95% CI:** 95% confidence interval.

5.4.6 Summary of conclusions from unpublished data in Chapter 5

Research Paper 2 presents results from the LAVIISWA baseline survey. Data presented in section 5.4 are unpublished, and were obtained from the LaVIISWA allergy outcomes survey, the urban survey and the asthma case-control study. Below is a summary of what these extra data add to Chapter 5:

a. Despite reduction in *S. mansoni* intensity (shown in **Research Paper 7, Appendix 3,** this thesis), community-wide intensive versus standard anthelminthic treatment in the LaVIISWA outcome survey had no effect on total, SWA-, SEA- and allergen extract-specific IgE and IgG4 profiles.
b. The general pattern of associations between antibody responses, *S. mansoni* infection and allergy-related outcomes among LaVIISWA baseline survey participants mirrored observations among LaVIISWA outcome survey and the urban survey participants
c. There were urban-rural differences in concentrations of total, allergen-, SEA- and SWA-specific antibodies. These were concomitant with urban-rural differences in helminth infection exposure and prevalence of allergy-related outcomes.

d. Findings that asthmatic schoolchildren had lower allergen-specific IgG4/ IgE ratios and total IgE / allergen-specific IgE ratios (compared to non-asthmatic controls) support the hypothesis that high IgG4/ IgE ratios and total IgE/ allergen-specific IgE ratios may be protective against clinical allergy.

CHAPTER 6. CARBOHYDRATE-SPECIFIC ANTIBODIES AND *SCHISTOSOMA MANSONI* INFECTION

6.1 Preamble

This chapter presents findings obtained using data from the rural and urban survey (described in Chapter 3) to assess associations between helminth (*S. mansoni*) infection (and intensity) and IgE and IgG responses to classical cross-reactive carbohydrate determinant (CCD) epitopes (**thesis objective 3**). Results are presented in Research paper 3 (below), titled "Microarray assessment of N-glycan-specific IgE and IgG profiles associated with *Schistosoma mansoni* infection in rural and urban Uganda". This manuscript has been accepted for publication as an original article in *Scientific Reports.*

There are important similarities between helminth antigens and common environmental allergens, key among them presence of non-mammalian core β-1,2-xylose and α-1,3-fucose substitutions on N-glycans expressed by schistosome, invertebrate and plant proteins. Immune responses to schistosomes are shaped to a great extent by the *Schistosoma* glycome. Responses to non-mammalian core modified N-glycans may impact schistosome and/or allergy diagnostics, or be utilised in interventions against schistosome infection or allergy. Furthermore, profiling these responses could be useful in understanding mechanisms of schistosome-allergy associations. Detailed research, including characterisation of these responses at a population level, is needed to determine their role and potential applications.

The current chapter maps associations of core β-1,2-xylose and core α-1,3-fucose with *S. mansoni* infection (and intensity) and the urban-rural environment in Uganda, using glycan microarray plasma IgE and IgG binding studies. This chapter further discusses the importance of glycan-specific antibodies in immunity to schistosome infection, diagnostics and cross-reactivity, serving as an important prologue to Chapter 7, which builds upon this thesis' overarching hypothesis (that helminth-induced antibodies

influence allergy profiles) to further postulate a key role for IgE to cross-reactive carbohydrate determinants in the epidemiology of allergy in the tropics.

6.2 Research Paper 3: Microarray assessment of N-glycan-specific IgE and IgG profiles associated with *Schistosoma mansoni* infection in rural and urban Uganda

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SECTION A – Student Details

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Thesis Title	HELMINTH-ALLERGY ASSOCIATIONS IN RURAL AND URBAN UGANDA: INSIGHTS FROM ANTIBODY STUDIES

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	SCIENTIFIC REPORTS
Please list the paper's authors in the intended authorship order:	Gyaviira Nkurununngi, Angela van Diepen, Jacent Nassuuna, Richard E Sanya, Margaret Nampijja, Irene Nambuya, Joyce Kabagenyi, Sonia Serna, Niels-Christian Reichardt, Ronald van Ree, Emily L Webb, Alison M Elliott, Maria Yazdanbakhsh, Cornelis H Hokke
Stage of publication	**In press**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I contributed to the conception and experimental design of the study. I conducted the experiments. I analysed the data and wrote the manuscript.

Student Signature: [redacted] Date: 14th FEB 2019

Supervisor Signature: [redacted] Date: 15/2/2019

Microarray assessment of N-glycan-specific IgE and IgG profiles associated with *Schistosoma mansoni* infection in rural and urban Uganda

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SUMMARY

Core β-1,2-xylose and α-1,3-fucose are antigenic motifs on schistosome N-glycans, as well as prominent IgE targets on some plant and insect glycoproteins. To map the association of schistosome infection with responses to these motifs, we assessed plasma IgE and IgG reactivity using microarray technology among Ugandans from rural *Schistosoma mansoni* (*Sm*)-endemic islands (n=209), and from proximate urban communities with lower *Sm* exposure (n=62). IgE and IgG responses to core β-1,2-xylose and α-1,3-fucose modified N-glycans were higher in rural versus urban participants. Among rural participants, IgE and IgG to core β-1,2-xylose were positively associated with *Sm* infection and concentration peaks coincided with the infection intensity peak in early adolescence. Responses to core α-1,3-fucose were elevated regardless of *Sm* infection status and peaked before the infection peak. Among urban participants, *Sm* infection intensity was predominantly light and positively associated with responses to both motifs. Principal component and hierarchical cluster analysis reduced the data to a set of variables that captured core β-1,2-xylose- and α-1,3-fucose-specific responses, and confirmed associations with *Sm* and the rural environment. Responses to core β-1,2-xylose and α-1,3-fucose have distinctive relationships with *Sm* infection and intensity that should further be explored for associations with protective immunity, and cross-reactivity with other exposures.

INTRODUCTION

Schistosomiasis is second only to malaria as a parasitic cause of human morbidity, with over 230 million infections globally, the majority of which occur in tropical and subtropical sub-Saharan Africa[1-3]. Despite important strides in coverage of anthelminthic treatment, reductions in infection prevalence have only been modest[4-6], and the long struggle for a vaccine breakthrough continues[7]. The host immunological response to *Schistosoma* infection is shaped to a significant extent by schistosome surface-exposed and secreted glycans and glycoproteins. For example, anti-glycan antibody responses dominate the host humoral response to schistosome larvae and eggs[8-10] and *Schistosoma* soluble egg antigen (SEA)-mediated Th2-polarisation profoundly relies on glycosylation[11,12]. In a mouse model for periovular granuloma formation, periodate treatment of SEA-coated beads inhibited their granulomogenic activity[13], further demonstrating the functional relevance of glycan-specific responses in *Schistosoma*-mediated immunity and pathology. A better understanding of the human immune response to the *Schistosoma* glycome may be beneficial to the current drive towards identification of better *Schistosoma* diagnostic markers and potent vaccine candidates[14-18].

Current insights into the *Schistosoma* glycome, the most characterised among parasites, have been particularly aided by mass spectrometry-based (MS) studies[19-21]. Analysis of asparagine (N)-linked glycans expressed by schistosomes reveals two standout, non-mammalian substitutions[22,23] on the trimannosyl-chitobiose core ($Man_3GlcNAc_2$, conserved in all eukaryotes): an α-1,3-fucose (α3Fuc) linked to the asparagine-linked N-acetylglucosamine (GlcNAc) of the chitobiose component and a β-1,2-xylose (β2Xyl) linked to the β-mannose of the trimannosyl component[24] (**Figure 1**). These substitutions are also found on nematode glycans from *Haemonchus contortus* and *Caenorhabditis elegans*[25-28], and on invertebrate[29,30] and plant glycans[31-33], but have so far not been detected on glycans from other helminths prevalent in the tropics[19]. Detailed MS studies have neither detected core β2Xyl nor core α3Fuc modified N-glycans in adult

schistosome worms but both are present in miracidia and eggs, while cercariae express core β2Xyl but no α3Fuc on the core GlcNAc[19]. Other common alterations to the schistosome $Man_3GlcNAc_2$ core include addition of antennae composed of GalNAcβ1-4GlcNAc (LacdiNAc, LDN), GalNAcβ1-4(Fucα1-3)GlcNAc (fucosylated LacdiNAc, LDN-F) and Galβ1-4(Fucα1-3)GlcNAc (Lewis X, LeX) units. These antennary modifications are expressed in schistosomes (at all developmental stages, albeit with varying surface expression patterns)[34] but are rare in mammals[35], and occur variably in other helminth species[19].

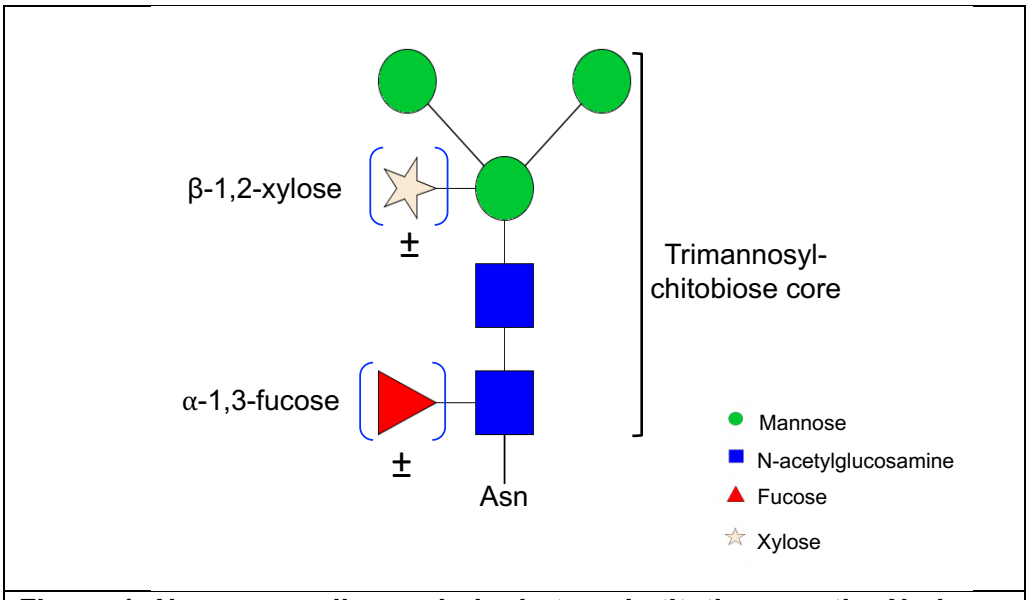

Figure 1. Non-mammalian carbohydrate substitutions on the N-glycan core. Non-mammalian monosaccharide substitutions are denoted by blue brackets. ± implies that motifs in brackets are present or absent in different species. Figure drawn using GlycoWorkbench software, version 2.1 (European Carbohydrates Database Project).

Core β2Xyl and α3Fuc modified schistosome egg N-glycoproteins induce potent Th2-type cellular responses[36]. In plants, core β2Xyl and α3Fuc may be the most common N-glycan epitopes targeted by human IgE[37,38]. It is plausible that N-glycan core substitutions play a major role in the glycan-dependent host response to chronic schistosomiasis. For example, most N-glycans on the SEA-derived glycoprotein omega-1 carry core α3Fuc motifs in combination with terminal LeX units[39]. Omega-1 drives both immunoregulatory[40] and Th2 responses[41], the latter in a glycan-dependent manner[12].

Kappa-5, another major component of the Th2-polarising SEA[42], expresses glycans modified with both core β2Xyl and core α3Fuc[43]. Whether protective immunity against *Schistosoma* infection and reinfection (long associated with host IgE responses[44,45]) can be credited to these epitopes will require further investigations in animal and human studies.

The advent of glycan microarray technology enabled serum/plasma profiling of antibodies raised to a wide repertoire of N-glycan variants during schistosome infections. This technology has been employed in a small number of human studies. Recently, in Ghana, sera from a few *S. haematobium* infected schoolchildren showed elevated IgE responses to core β2Xyl modified N-glycans on a synthetic glycan microarray[46], and in sera from a small cohort of *Schistosoma mansoni* (*Sm*)-infected children and adults near Lake Albert, Uganda, IgG1-4 subclass responses to core β2Xyl and α3Fuc motifs were examined using the same array[47]. Two other human studies employing shotgun microarrays constructed of complex native schistosome N-glycans showed strong anti-glycan IgG and IgM responses against a wider range of N-glycans during schistosome infections[48,49]. A better understanding of population-level immune responses to *Schistosoma* glycans is important for research and clinical applications, and requires larger, well-defined immuno-epidemiological studies in endemic settings.

Fishing villages in the Lake Victoria islands of Koome, Uganda, have a high prevalence of *Sm*[50-53], and have been surveyed as part of a portfolio of studies on helminth infections and allergy-related outcomes in Uganda. This setting provided a unique opportunity, within the context of a well-characterised large study[50], to correlate epidemiological trends pertaining to *Sm* infection (and intensity) with microarray-detected plasma IgE and IgG responses to N-glycans with and without core α-1,3-fucosylation and/or β-1,2-xylosylation. Plasma from residents of nearby mainland urban communities with lower *Sm* exposure enabled us to make rural-urban comparisons of anti-glycan antibody responses.

METHODS

Study design and population

Individuals included in the current investigation were randomly selected using a Stata program (StataCorp, College Station, USA) from participants of two cross-sectional surveys in rural and urban Uganda, who had a sufficient volume of stored plasma. The rural survey was the outcome survey (year three, September 2015 – August 2016) of the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA; ISRCTN47196031)[50], a cluster-randomised trial of community-based standard versus intensive anthelminthic intervention in 26 *Sm*-endemic fishing villages of Koome islands (Lake Victoria, Uganda). The trial description[50,53] and survey results after three years of intervention[52] have been published: briefly, standard intervention included annual, community-based, mass drug administration of praziquantel; intensive intervention included quarterly praziquantel. The urban survey (September 2016 – September 2017) was conducted in the 24 sub-wards of Entebbe municipality, an area with lower helminth exposure, located on the northern shores of Lake Victoria (approximately 35km from Koome). It was designed to collect data from an urban setting for comparison with the *Sm*-endemic rural survey.

In both surveys, intestinal helminth infections were assessed using the Kato-Katz (KK) method[54] on a single stool sample (prepared on two slides, read by different technicians). The remaining sample was stored at -80°C and later investigated for *Sm, Necator americanus* and *Strongyloides stercoralis* infections using multiplex real-time PCR[55,56]. Mid-stream urine was also assessed for *Sm* circulating cathodic antigen (CCA) using a point-of-care test (Rapid Medical Diagnostics, Pretoria, South Africa). *Schistosoma haematobium* is not present in the surveyed areas[57]. Blood samples were processed to obtain plasma for immunological measurements, including N-glycan-specific IgE and IgG by microarray (detailed below) and *Schistosoma* egg [SEA]- and adult worm [SWA] antigen-specific IgE, IgG4 and IgG by ELISA (supplementary material).

The research ethics committees of the Uganda Virus Research Institute and the London School of Hygiene and Tropical Medicine, and the Uganda National Council for Science and Technology approved this work. All methods were performed in accordance with guidelines and regulations of these committees. Informed consent was obtained from all participants and/or their legal guardians and assent from children aged ≥ 8 years.

Microarray detection of N-glycan-specific IgE and IgG

Immunoglobulin E and G responses to 135 chemically synthesised glycans with and without core α-1,3-fucosylation and, or, β-1,2-xylosylation (supplementary **Figure S1**) were assessed using a non-commercial microarray. Fluorescently-labeled bovine serum albumin (BSA) was included as an array printing control. Microarray construction procedures have been described in detail elsewhere[48,58]. The glycan antibody binding assay was adapted from existing procedures[17,46,49,59], as follows: Nexterion H N-hydroxysuccinimide-coated microarray slides (Schott AG, Mainz, Germany) (pre-blocked with 50mM ethanolamine in 50mM sodium borate buffer pH 9.0, and stored at -20°C) were thawed at room temperature (RT) and covered with silicone gaskets to create seven wells with printed microarrays per slide. Each microarray was incubated with 300 µl of a 1:30 plasma dilution in 1% BSA - 0.01% Tween20 for one hour at RT while shaking. After sequential washes with PBS-0.05% Tween20 and PBS, the slides were incubated for 30 minutes at RT in the dark with PromoFluor 647-labelled anti-human IgE (diluted 1/150 in PBS-0.01% Tween20) and Cy3-labelled anti-human IgG (diluted 1/1000 in PBS-0.01% Tween20), while shaking. After a final wash with PBS-0.05% Tween20, PBS and deionised water, sequentially, the slides were dried and kept in the dark until scanning. The slides were scanned for fluorescence at a 10µm resolution with a G2565BA scanner (Agilent Technologies, CA, USA) using 633nm and 532nm lasers for detection of reactivity to glycan-specific IgE and IgG, respectively.

Data analysis

Using GenePix Pro 7.0 software (Molecular Devices, CA, USA), a spot-finding algorithm

was used to align and re-size fluorescence spots in the microarray images, without setting a composite pixel intensity threshold. Data on median fluorescence intensity (MFI) for each spot and the local background were then exported to Microsoft Excel software, where background MFI subtraction was done for each glycan structure, averaged over four spots. Further processing of IgG and IgE MFIs in Excel was done as described by Oyelaran *et al.*[60] and Amoah *et al.*[46], respectively, to yield $\log_2$-transformed values.

Graphical representations of antibody responses and further data analyses were done using Stata 13.1 (College Station, Texas, USA), R (R foundation for Statistical Computing, Vienna, Austria) via the RStudio interface (version 1.1.383, RStudio, Inc. Boston, USA) and GraphPad Prism (version 6.0e, Fay Avenue, La Jolla, CA, USA). *Schistosoma mansoni* infection and the rural-urban environment were the main exposures of interest: we compared anti-glycan antibody responses between *Sm* infected and uninfected participants separately in the rural and urban survey, and thereafter between rural and urban participants. Initial analyses considered each anti-glycan antibody response independently, while further analyses combined antibody responses to reduce the dimensionality of the outcome data, as detailed below.

Rural-urban differences in *Sm* prevalence and *Schistosoma*-specific antibodies were assessed using survey design-based logistic and linear regression, respectively. Most $\log_2$-transformed anti-glycan IgE responses maintained a skewed distribution. Therefore, Mann-Whitney tests were used to assess differences in individual glycan structure-specific antibody responses between *Sm* infected and uninfected participants and between rural and urban participants. Most $\log_2$-transformed anti-glycan IgG responses were normally distributed and were assessed using unpaired t tests. The Kruskal-Wallis (IgE responses) and one-way ANOVA test (IgG responses) were also conducted to assess differences along the infection intensity gradient. Since many of the anti-glycan antibody responses were correlated, the above tests were conducted within a Monte

Carlo simulation approach based on 1000 permutations, to generate empirical p-values corrected for multiple testing.

Given the large number of outcomes, two data reduction techniques were used to investigate associations between exposures and outcomes. First, principal component analysis (PCA) was run in Stata to transform groups of correlated anti-glycan responses into fewer, uncorrelated artificial variables (principal components, PCs), which were then compared by 1) survey setting, and 2) *Sm* infection and intensity status using survey design-based linear regression. Second, unsupervised hierarchical clustering analysis (HCA, complete linkage using Euclidean distance) was conducted in R to further identify homogeneous sets of N-glycan-specific responses. The resultant IgE and IgG clusters were then assessed for associations with survey setting and *Sm* infection using the global test[61-63] executed in R with the Globaltest package (version 5.33.0).

RESULTS

Characteristics of the rural and urban survey participants included in this analysis are presented in **Table 1.** Rural participants were, on average, older [median age (IQR) 22 (5, 37)] than urban participants [median age (IQR) 11 (5, 18)] (p<0.001). A significantly higher percentage of rural, compared to urban participants, were infected with *Sm* (KK, p=0.002; PCR, p<0.001; CCA, p=0.015). Furthermore, median levels of total IgE (p<0.001) and SEA- and SWA-specific IgE (p<0.001), IgG4 (p=0.001) and IgG (p=0.002 and p<0.001, respectively) were higher among rural compared to urban participants.

Characteristic	**Rural** (n=209)	**Urban** (n=62)	**p value**
Age in years, median (IQR)	**22 (5, 37)**	11 (5, 18)	<0.001*
Male sex, n/N (%)§	97/209 (44.3)	18/62 (29.0)	0.163¶
Helminth infections, n/N (%)§			
S. mansoni (single KK)	**54/197 (34.5)**	4/48 (8.3)	0.002¶
S. mansoni intensity (KK)			
Uninfected (0 eggs/g)	143/197 (65.5)	**44/48 (91.7)**	
Light (0-99 eggs/g)	**29/197 (17.2)**	3/48 (6.3)	
Moderate (100-399 eggs/g)	**14/197 (10.2)**	1/48 (2.1)	
Heavy (≥400 egg/g)	**11/197 (7.0)**	0/48 (0.0)	0.002¶
S. mansoni (PCR)	**77/196 (44.9)**	6/48 (12.5)	<0.001¶
S. mansoni (urine CCA)	**118/199 (66.0)**	21/58 (36.2)	0.015¶
Any nematode infection#	**43/196 (18.7)**	1/48 (2.1)	0.001¶
Total IgE (kU/L), median (IQR)	**548.4 (404.4, 666.9)**	103.3 (63.8, 146.5)	<0.001*
***Schistosoma* egg and worm-specific antibody levels, (µg/ml), median (IQR)**			
SEA-specific IgE	**4.2 (2.6, 6.6)**	2.2 (1.4, 3.6)	<0.001*
SWA-specific IgE	**3.9 (2.4, 5.9)**	2.1 (1.3, 3.1)	<0.001*
SEA-specific IgG4	**161.0 (45.9, 663.8)**	8.8 (0.0, 48.4)	0.001*
SWA-specific IgG4	**71.6 (39.5, 188.1)**	32.1 (7.8, 57.9)	0.001*
SEA-specific IgG	**1687.3 (848.1, 2727.7)**	730.7 (527.4, 1413.4)	0.002*
SWA-specific IgG	**1432.4 (845.8, 1941.6)**	804.5 (572.7, 1311.3)	<0.001*

Table 1. Study participants: *Schistosoma mansoni* infection and *Schistosoma*-specific antibodies

§Percentages adjusted for survey design.

¶P values obtained from survey design-based logistic regression.

*P values obtained from survey design-based linear regression.

#Infection with any of Strongyloides stercoralis, Necator americanus (assessed by PCR), Trichuris trichiura, Ascaris lumbricoides (assessed by KK) and Mansonella perstans (assessed by modified Knott's method).

Percentages / medians that are significantly higher in one setting compared to the other (p≤0.05) are highlighted in bold.

KK: Kato-Katz; **PCR**: Polymerase Chain Reaction; **CCA**: Circulating Cathodic Antigen; **IQR**: Interquartile range; **SEA**: Schistosoma egg antigen; **SWA**: Schistosoma adult worm antigen

We recently reported that community-based intensive versus standard anthelminthic intervention in the rural survey reduced *Sm* infection intensity but had no effect on the overall *Sm* prevalence (measured using the urine CCA test)[52]. The current analysis found no evidence of an effect of intensive versus standard treatment on total IgE, SEA- or SWA-specific antibodies, or on antibody reactivity to any of the N-glycans on the microarray. Therefore, data from the rural survey were not stratified by trial treatment arm in the further analyses presented herein.

Associations between *S. mansoni* infection and IgE and IgG responses to individual core β-1,2-xylosylated and core α-1,3-fucosylated N-glycans

In the rural survey, IgE and IgG responses to the β2Xyl modified $Man_3GlcNAc_2$ core (G34) were significantly higher among *Sm* infected (KK and/or PCR, and CCA positive), compared to uninfected individuals, and were positively associated with *Sm* infection intensity (KK) [**Figure 2, a-f**] and SWA- and SEA-specific IgE and IgG (supplementary **Table S1)**. Observations were similar for the N-glycan core carrying both β2Xyl and α3Fuc (G37). However, IgE and IgG responses to the N-glycan core carrying α3Fuc only (G73) were similar between *Sm* infected and uninfected rural individuals (**Figure 2, a-f**), but positively associated with SWA- and SEA-specific IgE and IgG (**Table S1**).

In the urban survey, IgE and IgG reactivity to core β2Xyl and/or core α3Fuc modified glycans was also higher in *Sm* infected (KK and/or PCR) compared to uninfected individuals, although differences were not statistically significant (**Figure 2, g-j**). However, IgE reactivity to core α3Fuc and core α3Fuc + core β2Xyl modified glycans was significantly positively associated with SEA- and SWA-specific IgE (**Table S1**).

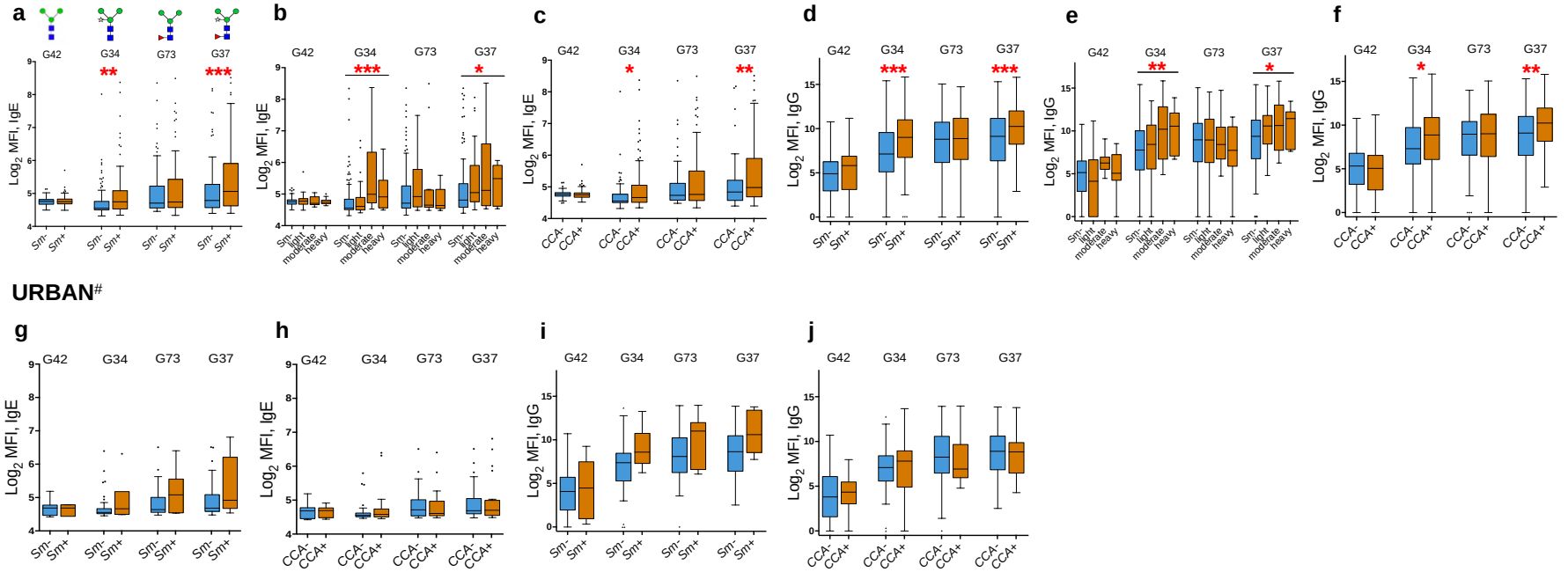

Figure 2. Associations between S. mansoni infection and IgE and IgG reactivity to N-glycans carrying non-mammalian core modifications.

Plasma from S. mansoni infected and uninfected rural and urban individuals were assessed for IgE and IgG reactivity to N-glycan structural variants with and without α-1,3-fucosylation and β-1,2-xylosylation, on a microarray platform. Box-and-whisker plots show background-subtracted and log2-transformed median fluorescence intensities (MFI) representing IgE (**a, b, c, g, h**) and IgG (**d, e, f, i, j**) reactivity to the $Man_3GlcNAc_2$ core structure (G42) and to α3Fuc- and/or β2Xyl-carrying $Man_3GlcNAc_2$ core structures (G34, G73 and G37). The plots show a horizontal line denoting the median, a box indicating the interquartile range (IQR), and whiskers drawn using the Tukey method (1.5 times IQR). Outliers (greater than 1.5 times IQR away from the median) are plotted as individual points. Mann-Whitney (IgE responses) and unpaired t test (IgG responses) were conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations (in order to adjust for multiple testing), to assess differences between infected and uninfected individuals. The Kruskal-Wallis (IgE responses) and one-way ANOVA test (IgG responses) were also conducted using the permutation approach to assess differences along the infection intensity gradient (**b** and **e**) in the rural survey.

#Infection prevalence and intensity was relatively low in the urban survey so analysis by Sm intensity is not shown for the urban setting.

*p<0.05; **p<0.01; ***p<0.001.

Sm: S. mansoni infection determined by detection of eggs in a single stool sample by Kato-Katz and/or PCR (rural infected n=84, uninfected n=113; urban infected n=6, uninfected n=42);

CCA: S. mansoni infection determined by a positive urine circulating cathodic antigen (CCA) result (rural infected n=118, uninfected n=81; urban infected n=21, uninfected n=37).

In the rural survey, *Sm* infection prevalence (and intensity) and median levels of *Schistosoma*-specific antibodies (except SEA-IgE) and β-1,2-xylosylated glycan (G34)-specific IgE and IgG were highest among 10-14-year old individuals (**Figure 3**). However, IgE and IgG reactivity to glycans carrying either core α3Fuc (G73) or both core β2Xyl and core α3Fuc (G37) peaked earlier (in the 5-9-year age group), akin to SEA-specific IgE. Age-stratified antibody reactivity patterns were less clear in the urban survey.

Immunoglobulin E and G responses to other N-glycan structural variants with core β2Xyl or both core β2Xyl and core α3Fuc[48] were also higher in *Sm* infected versus uninfected individuals (**Figure S2**). IgE and IgG reactivity to non-xylosylated and non-fucosylated glycans was not associated with *Sm* infection (data not shown), except for those glycans with antennae constructed of LDN-F (G90) and LeX (G89) units (**Figure S3**).

Infection with other helminths, malaria or HIV was not associated with IgE or IgG reactivity to any glycans on the microarray (data not shown).

Principal component analysis of anti-glycan antibody responses

Antibody responses to individual core modified N-glycans were strongly correlated. Principal component analysis (PCA) was conducted to summarise these responses, and to evaluate to what extent the resultant principal components (PCs) were associated with *Sm* infection.

Scatterplots of PC1 and PC2 loadings are shown in **Figure 4**. In the rural survey, the first two IgE and IgG PCs each accounted for 37% of the total variance in the data (IgE: PC1 28.2%, PC2 8.8%; IgG: PC1 27.7%, PC2 9.7%). Principal component 1 was characterized by responses to core β2Xyl and/or α3Fuc modified glycans while PC2 was characterized by responses to non-xylosylated and non-fucosylated glycans (**Figure 4, panel a** and **b**). Scores for IgE PC1, but not PC2, were higher among *Sm* infected (KK or PCR) compared to uninfected individuals (crude p=0.028, age- and sex-adjusted

p=0.167). Similarly, IgG PC1 scores were higher among *Sm* infected (KK or PCR) compared to uninfected individuals (crude p=0.009, adjusted p=0.027). There were no differences in PC scores between CCA+ and CCA- individuals.

In the urban survey, the first two IgE and IgG PCs accounted for 31% and 35% of the total variance, respectively (IgE: PC1 19.4%, PC2 11.5%; IgG: PC1 24.2%, PC2 10.6%). Interestingly, most IgE responses to glycans carrying core β2Xyl without α3Fuc clustered with non-xylosylated and non-fucosylated glycans in PC2 while responses to glycans carrying both core β2Xyl and α3Fuc and those carrying core α3Fuc without β2Xyl clustered together in PC1 (**Figure 4, panel c**). Akin to the rural survey, scores for IgE and IgG PC1 were higher among *Sm* infected compared to uninfected urban individuals.

Scores for PC1 were positively associated with SWA- and SEA-specific IgE and IgG in both surveys, while PC2 scores were inversely associated with the same *Schistosoma*-specific antibodies (**Table S1**).

In addition to PCA, we conducted HCA to further identify groups of anti-glycan IgE and IgG responses that might be jointly elicited in *Sm* infected versus uninfected individuals. **Figure S4** shows clusters of IgE and IgG responses in the rural and urban survey, and the dominant core substitutions on the glycans in these clusters. Generally, antibody clusters comprising core β2Xyl modified glycans were positively associated with *Sm* infection and intensity in both surveys (**Table S2**).

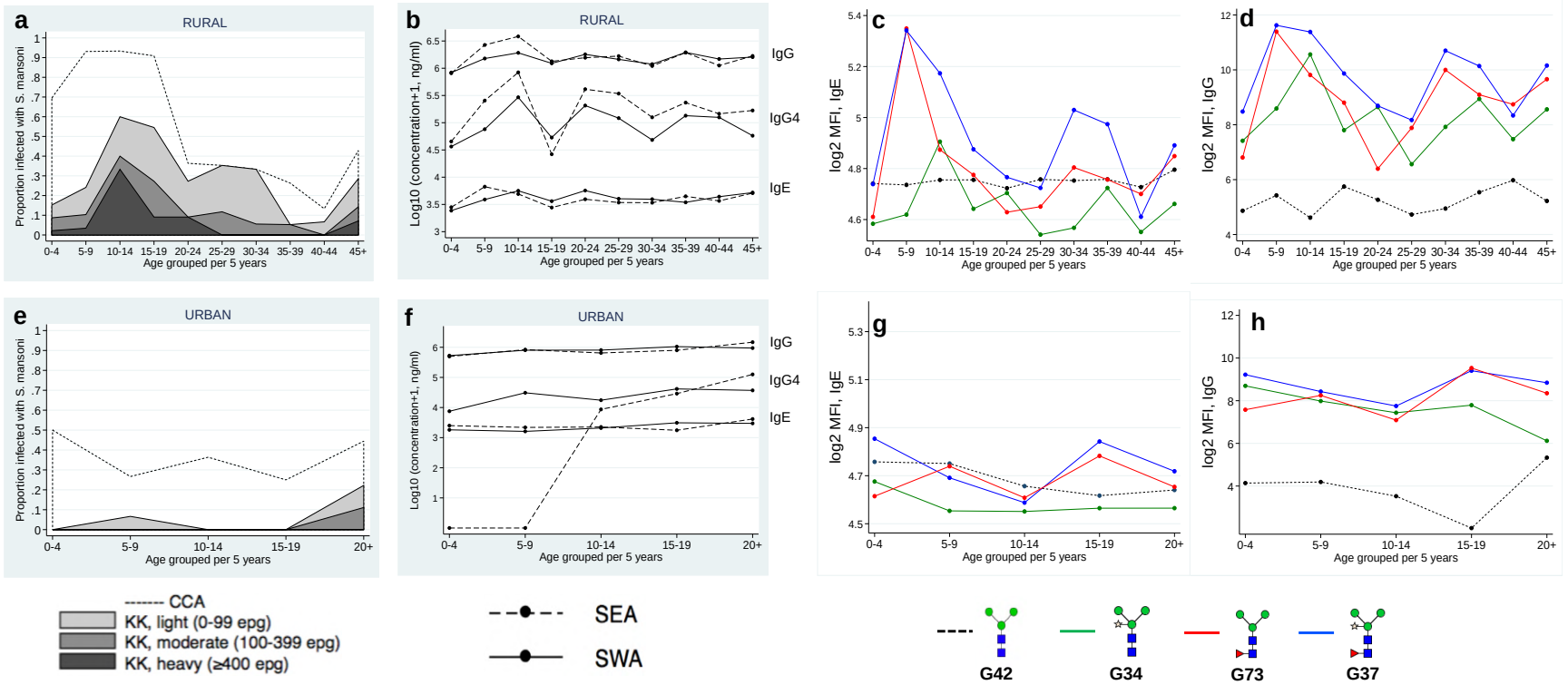

Figure 3. Age-stratified prevalence of S. mansoni infection and median IgE and IgG reactivity to SWA, SEA and α-1,3-fucosylated and β-1,2-xylosylated N-glycans. **(a)** and **(e):** rural and urban prevalence and intensity of S. mansoni infection, by age group. **(b)** and **(f)**: Median IgG, IgG4 and IgE reactivity to SWA and SEA, by age group, among rural and urban participants, respectively. **(c)** and **(g)**: Median IgE reactivity to the $Man_3GlcNAc_2$ core and to α3Fuc- and/or β2Xyl modified $Man_3GlcNAc_2$ core structures, by age group, among rural and urban participants, respectively. Plotted results are from all participants, irrespective of Sm infection status. **(d)** and **(h):** Median IgG reactivity to the $Man_3GlcNAc_2$ core and to α3Fuc- and/or β2Xyl-modified $Man_3GlcNAc_2$ core structures, by age group, among rural and urban participants, respectively. Plotted results are from all participants, irrespective of Sm infection status.

CCA: S. mansoni infection determined by a positive urine circulating cathodic antigen (CCA) result; **KK**: S. mansoni infection determined by detection of eggs in a single stool sample by Kato-Katz (KK); **epg**: eggs per gram of stool; **SWA**: Schistosoma adult worm antigen; **SEA**: Schistosoma egg antigen

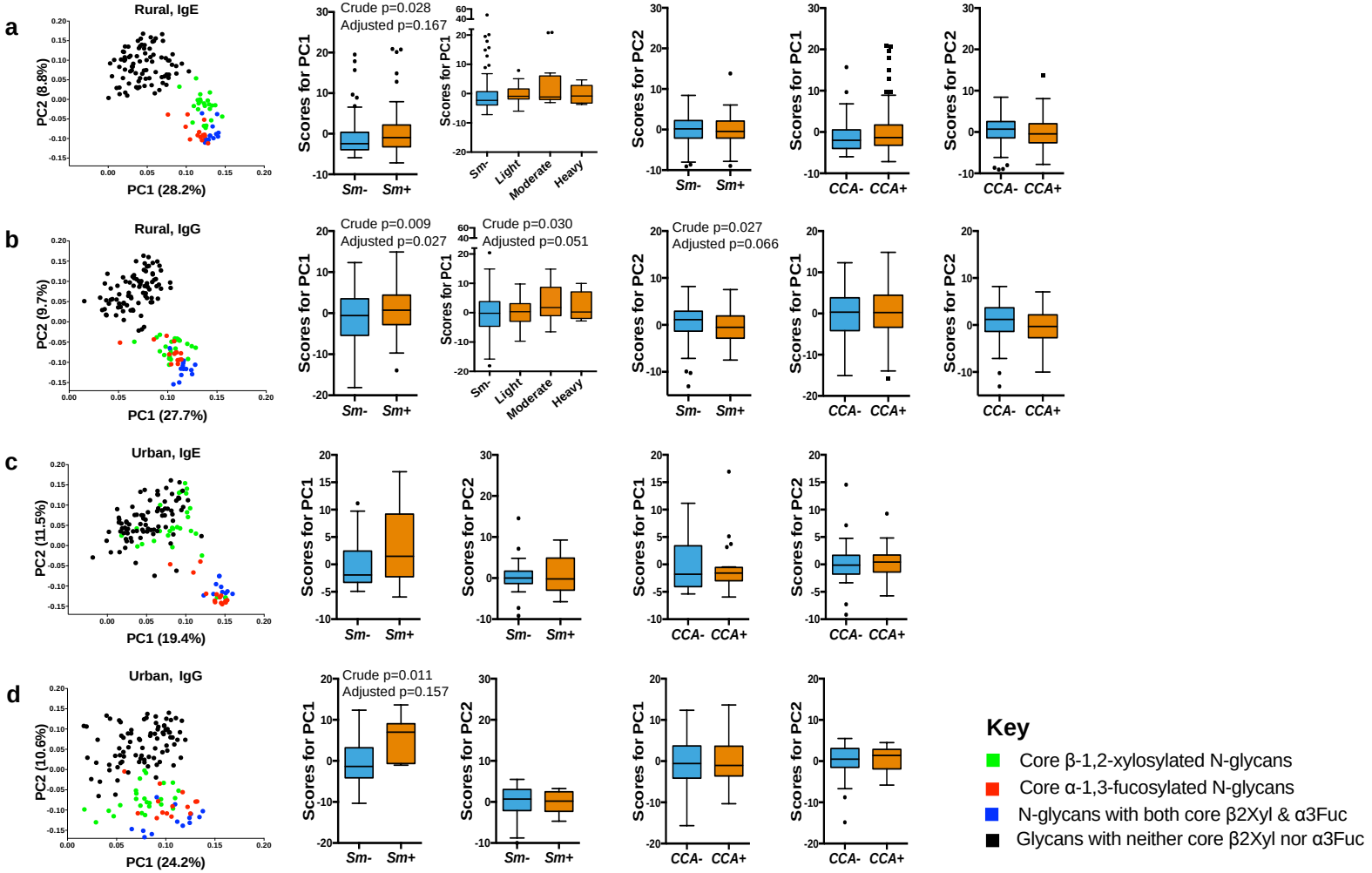

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Figure 4. Principal component analysis of anti-glycan antibody responses. Scatterplots of first (PC1) and second factor (PC2) loadings derived from principal component analysis of IgE and IgG responses to 135 synthetic N-glycans. Box-and-whisker plots show comparison of PC1 and PC2 scores between S. mansoni infected and uninfected individuals. The plots show a horizontal line denoting the median, a box indicating the interquartile range (IQR), and whiskers drawn using the Tukey method (1.5 times IQR). Outliers (greater than 1.5 times IQR away from the median) are plotted as individual points. Panels **a** and **b** show IgE and IgG profiles, respectively, among rural participants. Panels **c** and **d** show IgE and IgG profiles, respectively, among urban participants. Associations between factor loading scores and S. mansoni infection and intensity were assessed by linear regression analysis in Stata 13.1. Crude and age- and sex-adjusted p values are shown for significant associations. All analyses were adjusted for survey design using the 'svy' command in Stata. **PC1:** Principal Component 1; **PC2:** Principal Component 2; **Sm:** S. mansoni infection determined by detection of eggs in a single stool sample by Kato-Katz and/or PCR (rural infected n=84, uninfected n=113; urban infected n=6, uninfected n=42); **CCA:** S. mansoni infection determined by a positive urine circulating cathodic antigen (CCA) result (rural infected n=118, uninfected n=81; urban infected n=21, uninfected n=37).

Rural-urban comparisons of anti-glycan antibody responses

Immunoglobulin E responses to individual core β2Xyl and/or α3Fuc modified glycans were higher among rural compared to urban participants, as exemplified in **Figure 5a**. Principal component analysis of data combined from both surveys yielded distinct groups of anti-glycan responses (**Figure 5b** and **5f):** PC1 was characterized by responses to core β2Xyl and/or α3Fuc modified glycans while PC2 was characterized by responses to non-xylosylated and non-fucosylated glycans. Scores for IgE PC1 (**Figure 5c**), but not PC2 (**Figure 5d**), were higher among rural compared to urban individuals (p=0.002). Differences in IgG PC1 scores were not statistically significant. However, IgG PC2 scores were lower among rural compared to urban individuals (p=0.013).

Further assessment by HCA showed that clusters that comprised IgE responses to core β2Xyl and/or α3Fuc modified glycans (IgE-C1, IgE-C2 and IgE-C4; **Figure S5**) were positively associated with the rural setting (**Table S4**), while IgE-C3 (characterised by very low responses, raised against non-xylosylated and non-fucosylated glycans) was positively associated with the urban setting. Immunoglobulin G response clusters were generally similar between rural and urban settings, except for IgG-C7 which comprised responses to non-xylosylated and non-fucosylated glycans and was positively associated with the urban setting.

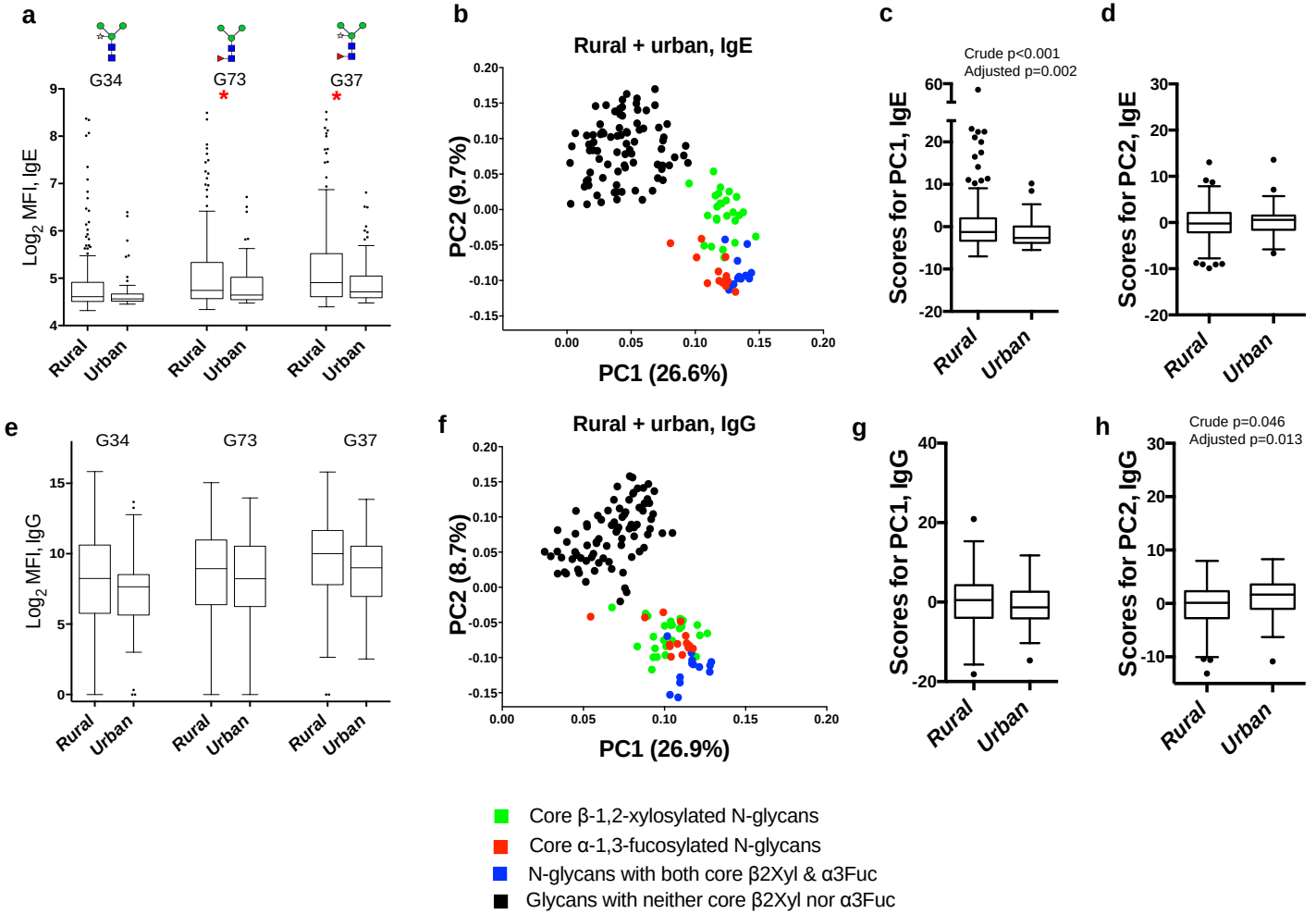

Figure 5. Rural-urban comparisons of anti-glycan antibody responses. (a, e): Box-and-whisker plots showing background-subtracted and log2-transformed median fluorescence intensities (MFI) representing IgE and IgG reactivity to individual α3Fuc- and/or β2Xyl-carrying $Man_3GlcNAc_2$ core structures in rural versus urban participants. The plots show a horizontal line denoting the median, a box indicating the interquartile range (IQR), and whiskers drawn using the Tukey method (1.5 times IQR). Outliers (greater than 1.5 times IQR away from the median) are plotted as individual points. Mann-Whitney (IgE responses) and unpaired t test (IgG responses) were conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations, to assess differences between rural and urban individuals. **(b, f):** First and second principal component loadings of N-glycan-specific

IgE and IgG responses among all participants, irrespective of survey setting. **(c, d, g, h)**: Box-and-whisker plots showing comparison of PC1 and PC2 scores between rural and urban individuals. Associations between PC scores and survey setting were assessed by linear regression in Stata 13.1. Crude and age- and sex-adjusted p values are shown for significant associations. All analyses were adjusted for survey design using the 'svy' command in Stata.
*p<0.05; **p<0.01; ***p<0.001.
PC1: Principal Component 1; **PC2:** Principal Component 2

DISCUSSION

By studying rural *Sm*-endemic Ugandan fishing communities and a proximate urban community, we have dissected antibody responses to core β2Xyl and α3Fuc modified N-glycans. Antibody responses to the core modified glycans were higher in the rural communities compared to a proximate urban community. In the urban community, IgE and IgG to both core β2Xyl and core α3Fuc were positively associated with *Sm* infection. In the rural communities, IgE and IgG to core β2Xyl were strongly positively associated with *Sm* infection while reactivity to core α3Fuc was elevated in both *Sm* infected and uninfected individuals. In the rural communities the concentration of antibodies to core α3Fuc modified N-glycans peaked ahead of the peak of *Sm* infection intensity, while the peak of antibodies to N-glycans with only core β2Xyl coincided with it.

The positive association between current *Sm* infection and IgE and IgG reactivity to N-glycans carrying only core α3Fuc in the urban, but not the rural communities, might reflect universal exposure to infection, and persistence of light infection despite treatment, in the rural setting. Core α3Fuc is abundant on N-glycans from *Sm* eggs but is not expressed by cercarial and adult worm N-glycans[19,20,24]. It is plausible that responses to core α3Fuc persist after active infection in high *Sm* exposure rural settings: in mice, eggs and hepatic granulomas persist long after clearance of worms[64]. Another explanation for elevated responses to α3Fuc in the rural communities, regardless of *Sm* infection status, is cross-reactivity. Core α-1,3-fucosylation and β-1,2-xylosylation are also present on certain plant and insect glycoproteins[29,30,65], hence similar core α3Fuc responses in both *Sm* infected and uninfected individuals may also be explained by an exposure other than schistosomes, more prevalent in the rural than the urban setting, that carries core α3Fuc. The observation that antibodies to core β2Xyl were significantly higher among *Sm* infected individuals in both urban and rural settings implies a dominant role for core β2Xyl (compared to core α3Fuc) in *Sm*-specific humoral immunity, shown

here for the first time. It also appears that responses only to core β2Xyl are more responsive to change in *Sm* exposure: core β2Xyl is abundant on cercarial N-glycans despite being absent in adult worms.

The prominent contribution of core β2Xyl and α3Fuc to cross-reactivity between schistosomes and other environmental exposures such as pollen, hymenoptera venom and vegetable foods[22,37,38] is a caveat against the use of core modified glycans in schistosome diagnostic tests. Cross-reactivity with other helminth infections might also occur, but only a few other helminth species[25-28], none of which are prevalent in humans in our survey settings, have so far been demonstrated to express glycans with core β2Xyl and α3Fuc motifs. More extensive glycomic studies of other helminths in our survey settings (*S. stercoralis,* hookworm, *T. trichiura, A. lumbricoides, M. perstans*) are warranted. However, we did not find any significant associations between these infections and IgE or IgG reactivity to core modified glycans.

Our observations that IgE and IgG reactivity to N-glycans modified with antennae carrying LDNF and LeX units were associated with *Sm* infection in both surveys are consistent with previous studies in animal models and in humans[49,66]. No associations were observed with responses to glycans carrying unsubstituted LDN units.

Principal component analysis indicated strong correlations between antibody responses to β-1,2-xylosylated glycans and responses to α-1,3-fucosylated glycans in both surveys. Core β2Xyl and α3Fuc epitopes can be found on similar *Sm* antigens, where they may be expressed on the same glycoproteins and glycans (such as those expressed by SEA)[19], inducing analogous immune responses[36]. Non-xylosylated and non-fucosylated glycans with antennae constructed of LDN, LDN-F or LeX units may be expressed on the same *Sm* antigens as glycans with core β2Xyl and α3Fuc motifs. Furthermore, these terminal antennary substitutions can occur on the same glycans as core β2Xyl and α3Fuc[19,39,43]. However, PCA showed that responses to non-core-substituted glycans with

LDN, LDN-F or LeX units did not cluster with core β2Xyl/α3Fuc substituted glycans. Temporal changes in expression of glycans on *Sm* antigens have been reported[20,34]; it is possible that these two groups of glycans are expressed at varying magnitudes during *Sm* antigen maturation. Positive associations between *Sm* infection and the first principal component (representing responses to core β2Xyl and α3Fuc) reflect the important role of these core substitutions in the glycan-dependent host response to *Sm*. To further evaluate their contribution to the host immune response to *Sm*, it will be important to compare their antibody reactivity with that of other highly antigenic terminal motifs absent from glycans on the array used in this study, such as multi-fucosylated LDN motifs[59]. It is important to note that while IgG is abundantly detected to many schistosome glycans, and is triggered by *Sm* infection, only to core β2Xyl and α3Fuc modified glycans is IgE abundantly detected[46,47,67].

Notably in the urban survey, PCA showed that IgE responses to core β2Xyl modified glycans clustered with responses to non-xylosylated and non-fucosylated glycans (Figure 4c), and IgG responses to core β2Xyl and α3Fuc separated out less distinctly (Figure 4d) than in the rural survey. The observed β2Xyl clustering patterns may be attributed to the greater intensity of repeated exposure to schistosome cercariae (core β2Xyl) and sustained egg deposition (core β2Xyl and α3Fuc) among rural compared to urban participants. In other words, rural-urban differences in antibody responses to core modified glycans may be indicative of differences in the intensity of *Sm* infection and/or degree of exposure between the two settings. However, this study did not have sufficient power to assess statistical interactions between the rural and the urban setting. Rural-urban differences in antibody responses to core modified glycans may also be explained by exposures other than schistosomes (mentioned above), perhaps more prevalent in the rural than the urban setting; however, this is unlikely as we observed strong associations between *Sm* infection and reactivity to core modified N-glycans, particularly to those carrying core β2Xyl. It is also noteworthy that urban survey participants were

significantly younger than rural participants; however, this disparity did not seem to influence the observed rural-urban differences in anti-glycan responses, as observed from test statistics before and after adjusting for age.

One of the key challenges in schistosomiasis vaccine development is the risk of allergic (IgE) sensitisation to candidate vaccine antigens[68]. Glycans are attractive vaccine candidates because they are generally considered to be benign as allergenic determinants[69,70]. There are a few known exceptions, such as the galactose-α-1,3-galactose (α-1,3-gal) epitope (found in non-primate mammalian proteins, and shown to elicit severe allergy)[71], so assessment for any associations between IgE to antigenic *Sm* glycans and allergy-related phenomena are important. The case for consideration of core modified *Sm* glycans as *Schistosoma* vaccine candidates will also need definite proof for an association between reactivity to core modified glycans and protection from *Sm* infection/re-infection. Our data suggests that a protective role, if any, is more plausible for core β2Xyl than core α3Fuc: in the rural survey, antibody responses to core α3Fuc (G73 and G37, Figure 3C and 3D) peaked in childhood, prior to the *Sm* infection peak in early adolescence, while responses to core β2Xyl (G34) coincided with the *Sm* infection peak (preceding the more 'protected' period in adulthood). However, concrete evidence is required from further population and mechanistic studies exploring the role of *Sm* N-glycans in protective immunity. For example, it may be important to assess antibodies to these core modifications (and other antigenic terminal motifs) in re-infection study cohorts evaluating the immunological characteristics of individuals who are *Sm*-resistant following anthelminthic treatment.

In conclusion, we provide an immuno-epidemiological description of IgE and IgG responses to N-glycans in rural and urban Uganda, highlighting the significance of core β2Xyl and core α3Fuc to the glycan-dependent host immune response during chronic schistosomiasis. Moreover, our data imply that IgE and IgG responses to core β2Xyl and α3Fuc modified N-glycans have distinctive relationships with *Sm* infection and

intensity, which may reflect their different contributions towards protective immunity against *Sm* that need to be further explored using mechanistic animal and human studies.

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Author contributions

G.N, C.H.H, M.Y, A.M.E, R.v.R, and A.v.D contributed to the conception and experimental design of the study. A.M.E, R.E.S and M.N led the field and clinic procedures. S.S and N.C.R constructed the synthetic microarrays. G.N conducted the

microarray antibody binding experiments. G.N, J.N, J.K and I.N participated in establishing and conducting all other immunological and parasitological experiments. G.N analysed the data with important contributions from E.L.W and A.v.D. G.N wrote the manuscript, with all authors contributing to the interpretation of the results, and revision and approval of the final manuscript. G.N is the guarantor of the article.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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6.3 Supplementary information for Research paper 3 (also available in the article's online repository at https://www.nature.com/srep/)

6.3.1 Experimental methods

6.3.1.1 S. mansoni adult worm (SWA)- and egg (SEA)-specific IgE and IgG4 ELISA

All but the first 2 columns of 4HBX Immulon (Thermo Scientific, NY, USA) 96-well plates were coated with 50µl of SWA [8 µg/ml] or SEA [2.4 µg/ml] (purchased from Professor Michael J Doenhoff, University of Nottingham) in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer (0.1M, pH 9.6). Two-fold dilutions of human IgE (Calbiochem, Beeston, UK) or IgG4 (Sigma-Aldrich) standard, diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4ºC. Plates were washed with phosphate-buffered saline (PBS 1X)-tween 20 solution, blocked with 150µl of 1% skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4ºC with 50µl of plasma samples diluted 1/20 (IgE assay) or 1/200 (IgG4 assay) with 0.1% skimmed milk in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates overnight at 4ºC with 0.5µg/ml of biotinylated monoclonal mouse anti-human IgE or IgG4 (BD Pharmingen™). This was followed by a 1-hour incubation with a streptavidin-Horseradish Peroxidase (strep-HRP) conjugate (Mast Group Ltd, Bootle, UK), diluted 1/4000 with assay buffer, at RT. Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped after 30 minutes with 25µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgE or IgG4 concentrations (ng/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

6.3.1.2 S. mansoni adult worm (SWA)- and egg (SEA)-specific IgG ELISA

All but the first 2 columns of 4HX Immulon (VWR, UK, Cat No 735-0465) 96-well plates were coated with 50µl of SWA [8 µg/ml] or SEA [2.4 µg/ml] (purchased from Professor Michael J Doenhoff, University of Nottingham) in bicarbonate (Na_2CO_3 + $NaHCO_3$) buffer

(0.1M, pH 9.6). Two-fold dilutions of human IgG (Sigma-Aldrich), diluted in bicarbonate buffer, were added to the first 2 columns of each plate to form standard curves. The plates were then incubated overnight at 4ºC. Plates were washed with phosphate-buffered saline (PBS)-Tween 20 solution, blocked with 150µl of 1% skimmed milk diluted in PBS-Tween 20 at room temperature (RT), and incubated overnight at 4ºC with 50µl of plasma samples diluted 1/3000 with 0.1% skimmed milk in PBS-Tween 20 (assay buffer). Plates were washed and antibody binding detected by incubating the plates for 1 hour at RT with 0.5µg/ml of polyclonal rabbit anti- human IgG/HRP (Dako, Denmark). Plates were developed by addition of 100µl of o-phenylenediamine (Sigma-Aldrich) and reactions stopped after 30 minutes with 25µl of 2M Sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgG concentrations (ng/ml) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA).

6.3.2 Supplementary figures and tables

Figure S1. Synthetic N-glycan structural variants on the microarray

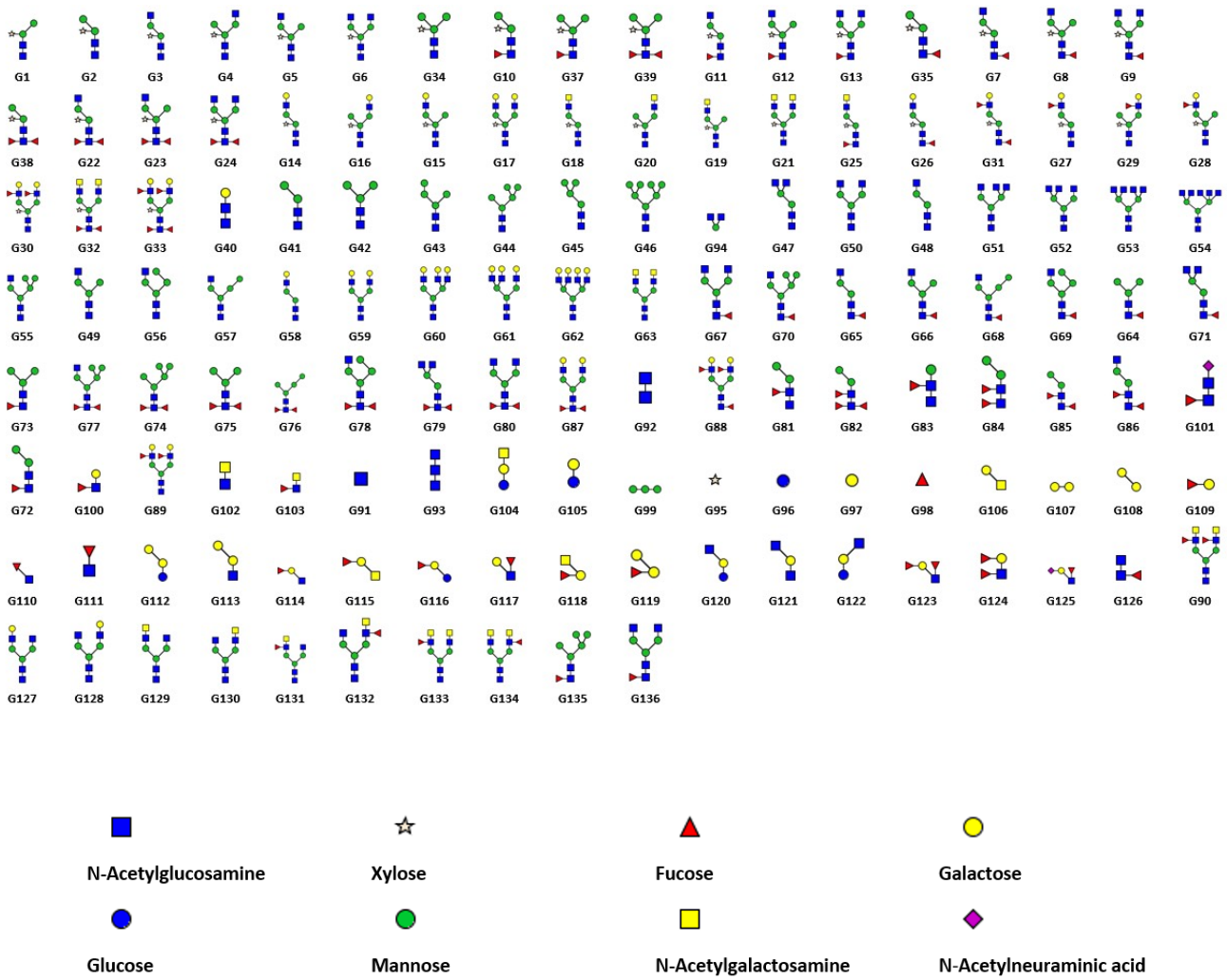
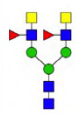


Table S1. Associations between anti-glycan and *Schistosoma* adult worm- and egg-specific antibody responses

		Associations between anti-glycan IgE & anti-Sm IgE				***Associations between anti-glycan IgG & anti-Sm IgG***			
	Glycan	**SEA-specific IgE**		**SWA-specific IgE**		**SEA-specific IgG**		**SWA-specific IgG**	
		β (95% CI)	*p*	*β (95% CI)*	*p*	*β (95% CI)*	*p*	*β (95% CI)*	*p*
RURAL		-0.02 (-0.07, 0.03)	0.364	-0.00 (-0.04, 0.04)	0.927	1.06 (-0.11, 2.24)	0.075	**3.06 (1.95, 4.16)**	**<0.001**
URBAN	G42	0.1 (0.0, 0.1)	0.050	0.1 (-0.2, 0.3)	0.660	0.48 (-2.05, 3.01)	0.678	-0.68 (-4.36, 3.01)	0.688
RURAL		**0.76 (0.36, 1.16)**	**0.001**	**0.86 (0.60, 1.11)**	**<0.001**	**2.67 (0.67, 4.68)**	**0.011**	**5.66 (3.26, 8.07)**	**<0.001**
URBAN	G34	0.1 (-0.1, 0.3)	0.165	0.78 (-0.02, 1.58)	0.056	1.43 (-1.06, 3.92)	0.225	1.49 (-2.07, 5.05)	0.369
RURAL		**0.81 (0.36, 1.26)**	**0.001**	0.52 (-0.29, 1.33)	0.205	**1.61 (0.05, 3.16)**	**0.044**	**2.71 (0.66, 4.75)**	**0.012**
URBAN	G73	**0.25 (0.02, 0.47)**	**0.036**	**1.01 (0.31, 1.69)**	**0.009**	1.62 (-1.29, 4.54)	0.239	1.21 (-1.75, 4.18)	0.379
RURAL		**1.13 (0.52, 1.74)**	**0.001**	**1.07 (0.39, 1.74)**	**0.003**	**2.26 (0.44, 4.08)**	**0.017**	**4.55 (3.46, 5.65)**	**<0.001**
URBAN	G37	**0.29 (0.04, 0.56)**	**0.029**	**1.61 (0.88, 2.33)**	**0.001**	1.79 (-1.52, 5.11)	0.251	1.95 (-1.53, 5.45)	0.236
RURAL		-0.01 (-0.11, 0.08)	0.762	0.01 (-0.09, 0.11)	0.865	0.32 (-0.11, 0.75)	0.137	**0.99 (0.19, 1.78)**	**0.016**
URBAN	G63	**0.05 (0.01, 0.11)**	**0.025**	0.15 (-0.16, 0.46)	0.316	0.36 (-1.30, 2.03)	0.632	0.55 (-1.83, 2.95)	0.609
RURAL		**0.04 (0.02, 0.05)**	**<0.001**	**0.05 (0.03, 0.08)**	**<0.001**	**1.39 (0.19, 2.59)**	**0.025**	**3.41 (2.09, 4.73)**	**<0.001**
URBAN	G90	-0.00 (-0.02, 0.01)	0.437	-0.00 (-0.04, 0.04)	0.890	**2.68 (0.97, 4.37)**	**0.006**	**3.24 (1.00, 5.47)**	**0.010**

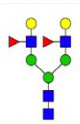
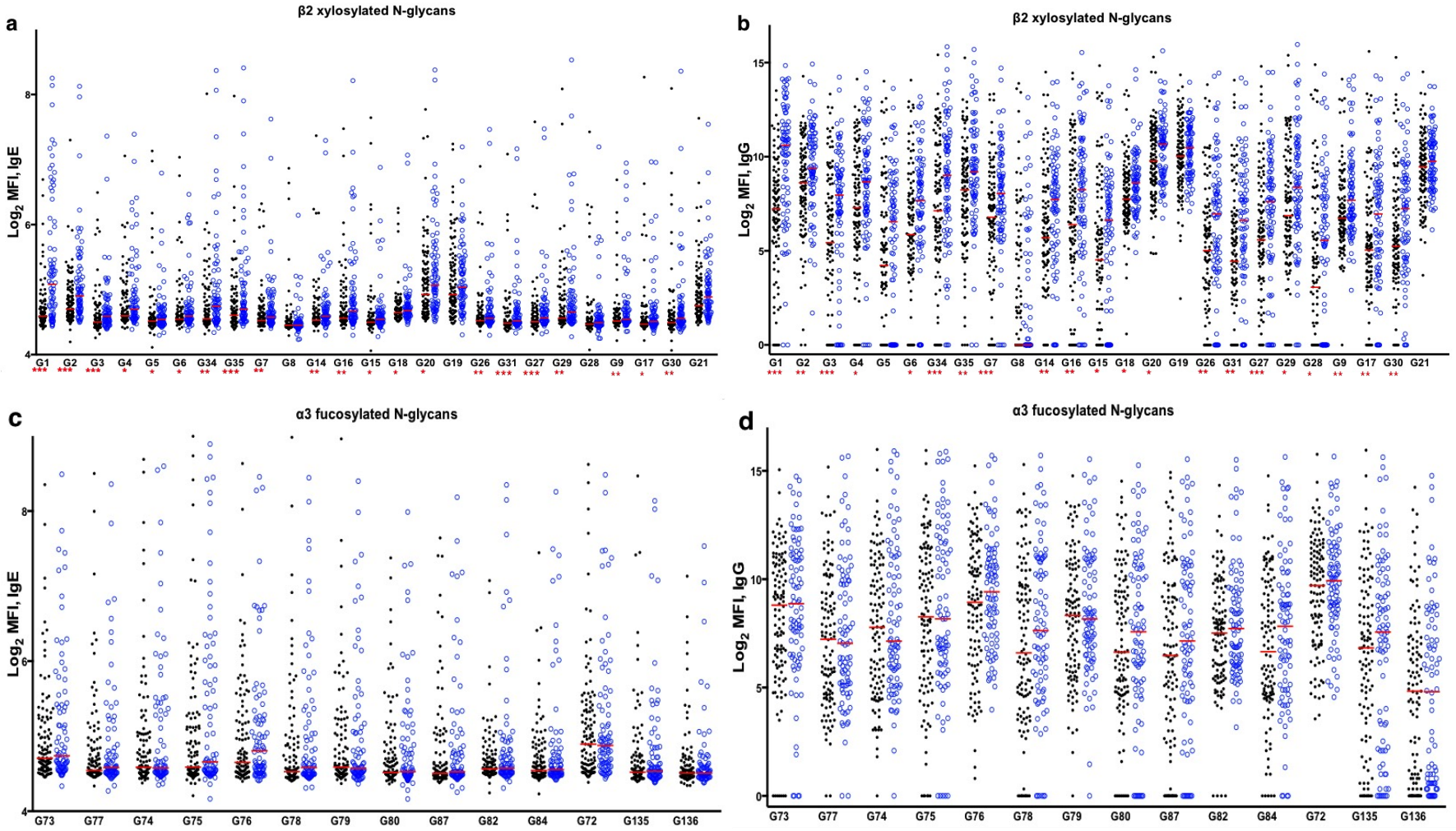
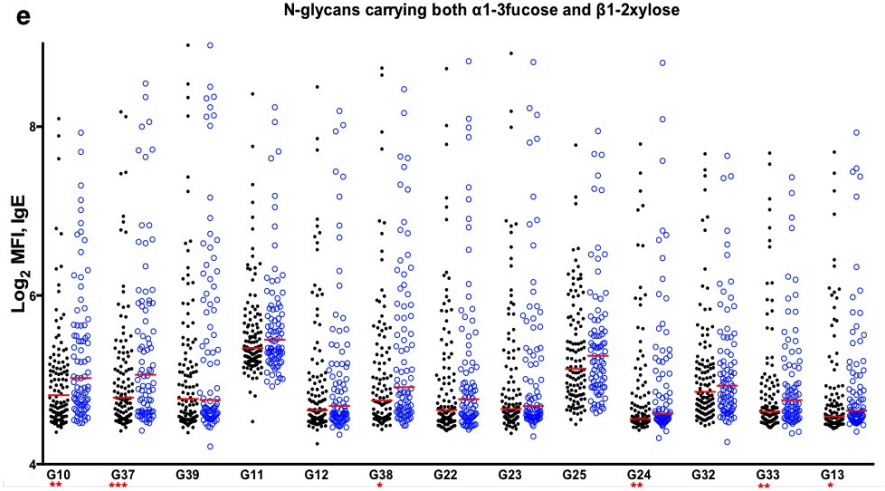
RURAL		0.04 (-0.03, 0.04)	0.813	0.01 (-0.04, 0.49)	0.815	1.24 (-0.04, 2.51)	0.057	**3.40 (1.11, 5.68)**	**0.005**
URBAN	G89	0.00 (-0.01, 0.01)	0.980	-0.04 (-0.09, 0.01)	0.118	**2.46 (0.33, 4.59)**	**0.028**	**3.51 (1.24, 5.77)**	**0.007**
RURAL	PC1	**6.51 (2.69, 10.34)**	**0.002**	**5.85 (1.80, 9.91)**	**0.006**	**4.27 (0.22, 8.33)**	**0.040**	**9.96 (6.75, 13.18)**	**<0.001**
URBAN		1.93 (-1.39, 5.25)	0.222	**9.48 (2.66, 16.31)**	**0.012**	5.02 (-4.49, 14.5)	0.263	5.88 (-3.58, 15.3)	0.193
RURAL	PC2	**-2.74 (-4.64, -0.83)**	**0.007**	-1.15 (-3.25, 0.92	0.263	**-1.82 (-3.41,-0.23)**	**0.027**	**-2.64 (-4.46, -0.81)**	**0.006**
URBAN		-1.89 (-4.33, 0.55)	0.114	-5.15 (-11.2, 0.86)	0.084	-0.89 (-2.94, 1.15)	0.347	-0.87 (-0.28, 1.15)	0.354

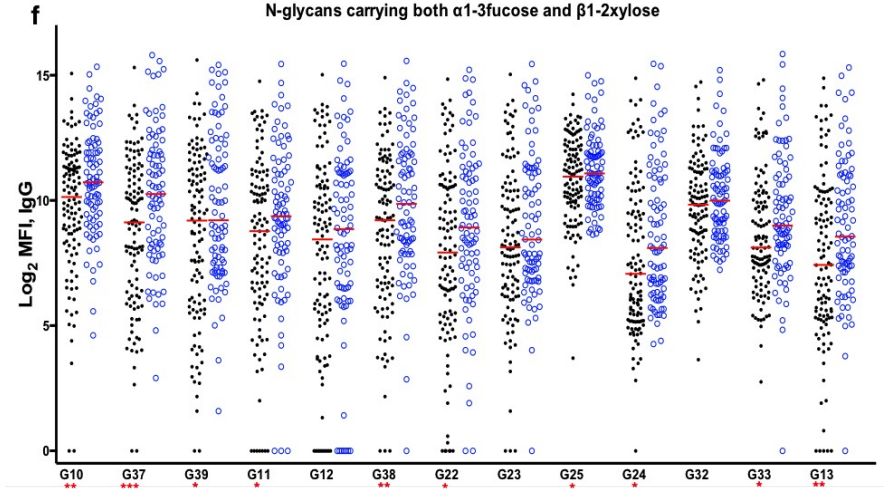
Table shows regression coefficients (β) and corresponding 95% confidence intervals (95% CI) and p-values for associations between anti-glycan antibody responses and *Schistosoma* adult worm (SWA)- and egg (SEA)-specific antibody responses, among rural and urban participants. The $Man_3GlcNac_2$ core structure (G42), α3fuc- and/or β2xyl-carrying $Man_3GlcNac_2$ core structures (G34, G73 and G37) and N-glycan core structures carrying LDN (G63), lLDNF (G90) and Lewis X (G89) antennae were chosen to represent the wide range of *Schistosoma*-associated N-glycans on the array. PC1 and PC2 denote first and second factor loadings derived from principal component (PC) analysis of IgE and IgG responses to the 135 synthetic N-glycans. PC1 was characterised by responses to core β2xyl and/or core α3fuc modified glycans while PC2 was characterised by responses to non-xylosylated and non-fucosylated glycans (figure 4 in main paper, panels a and b).
All associations are adjusted for the survey design using the 'svy' command in Stata 13.1, and for age and sex.

Figure S2. Associations between *S. mansoni* infection (KK and/or PCR) and IgE and IgG reactivity to N-glycan structural variants carrying α1-3 fucose and/or β1-2 xylose

RURAL SURVEY



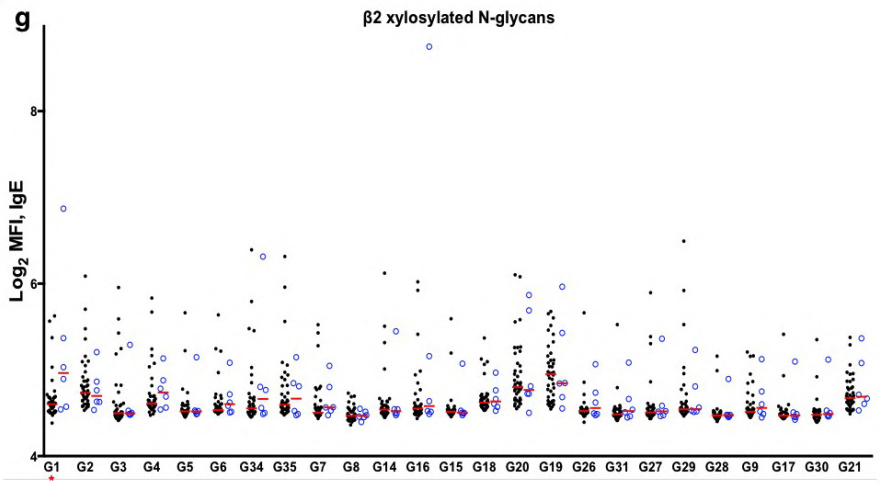


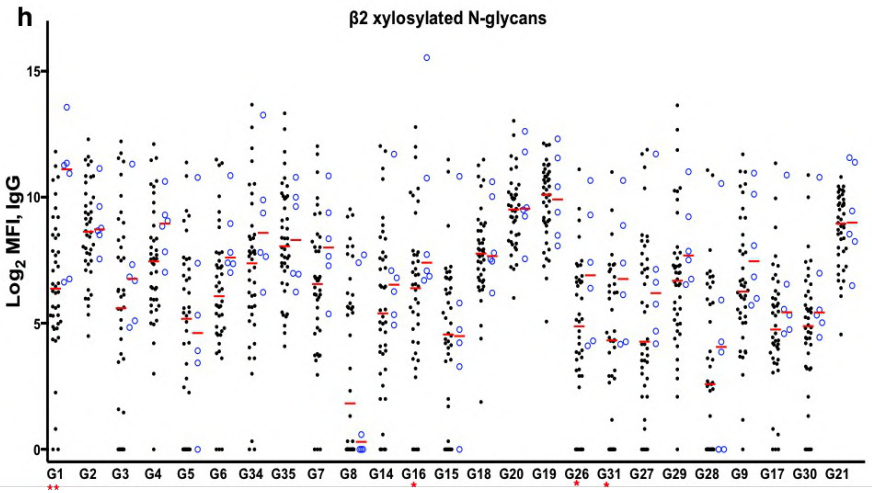


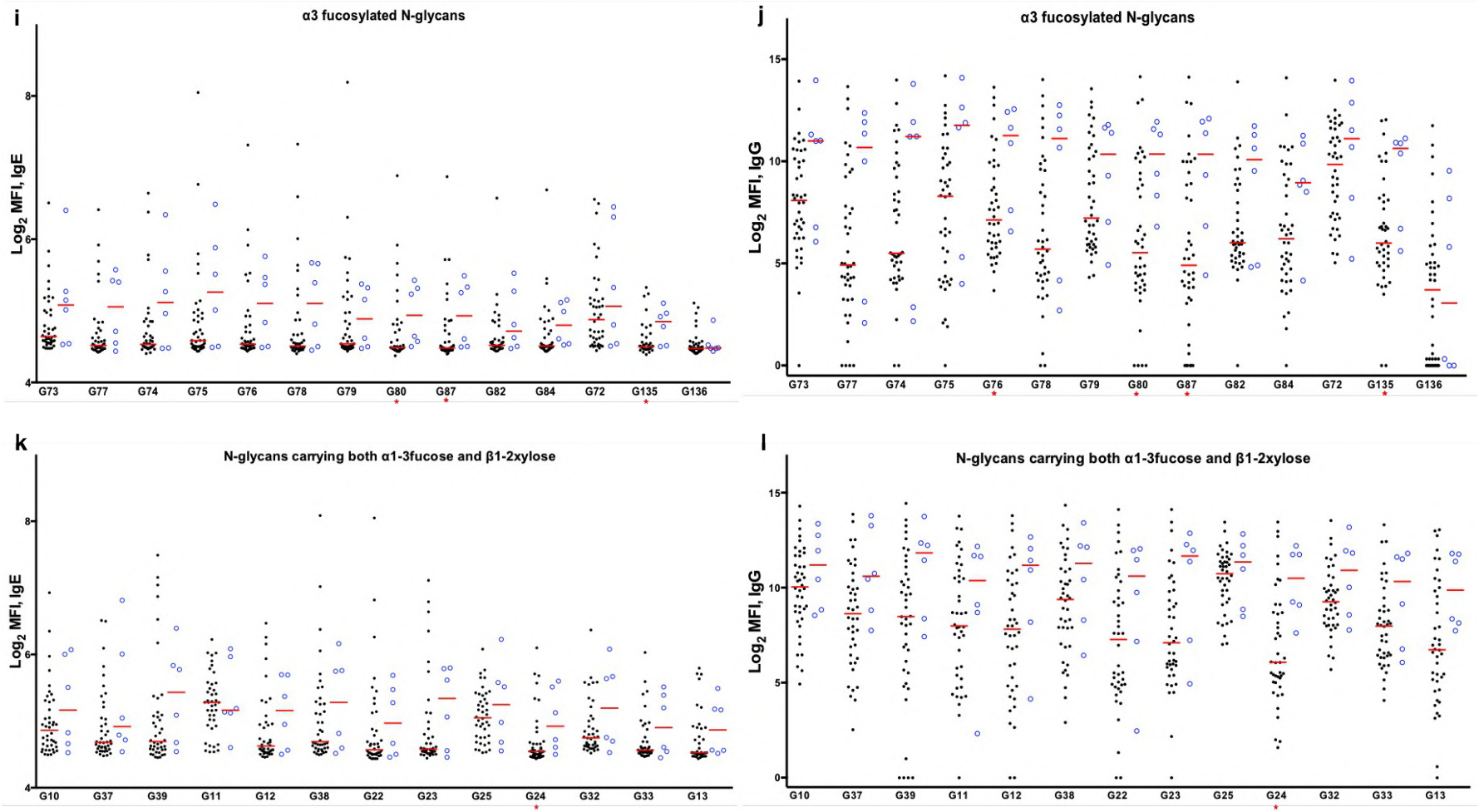
S. mansoni infected (KK and/or PCR, n=84)

Uninfected (n=113)

URBAN SURVEY





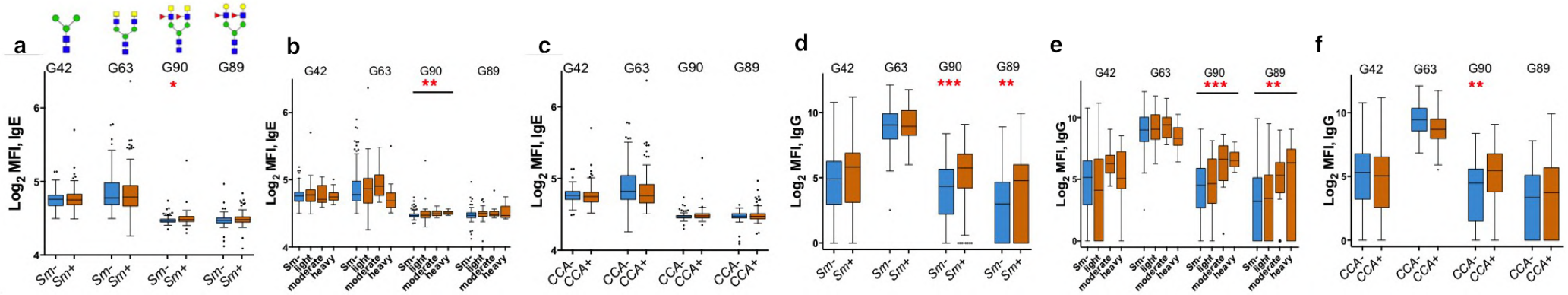


○ S. mansoni infected (KK and/or PCR, n=6)
● Uninfected (n=42)

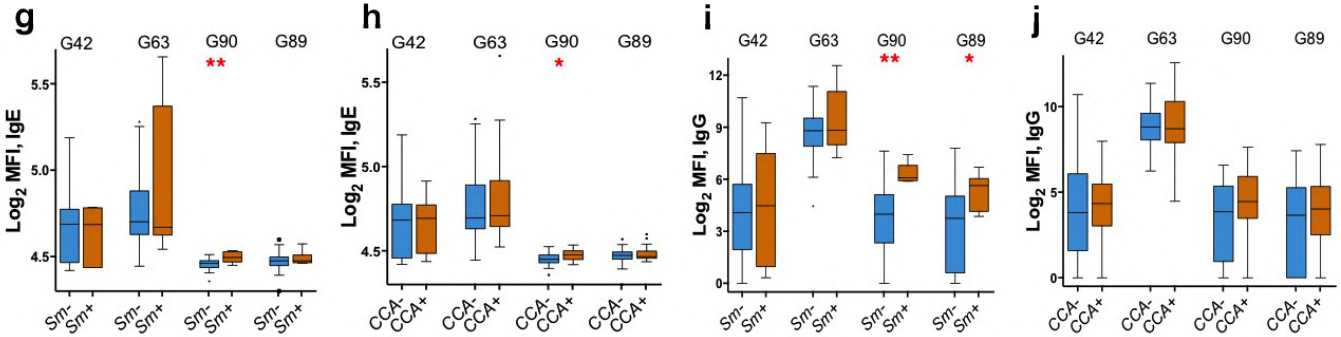
Figure shows background-subtracted and log_2-transformed median fluorescence intensities (MFIs) representing IgE (a, c, e, g, i, k) and IgG (b, d, f, h, j, l) reactivity to α-1,3-fucosylated and β-1,2-xylosylated N-glycans, among *S. mansoni* infected (KK and/or PCR) [open circles] and uninfected (closed circles) rural and urban individuals. Mann-Whitney (IgE responses) and unpaired t test (IgG responses) were conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations, to assess differences between infected and uninfected individuals.
*p<0.05; **p<0.01; ***p<0.001

Figure S3. Associations between *S. mansoni* infection and IgE and IgG reactivity to N-glycans carrying GalNAcβ1-4GlcNAc (LDN), GalNAcβ1-4(Fucα1-3)GlcNAc (LDNF) and Galβ1-4(Fucα1-3)GlcNAc (LeX) antennae

RURAL

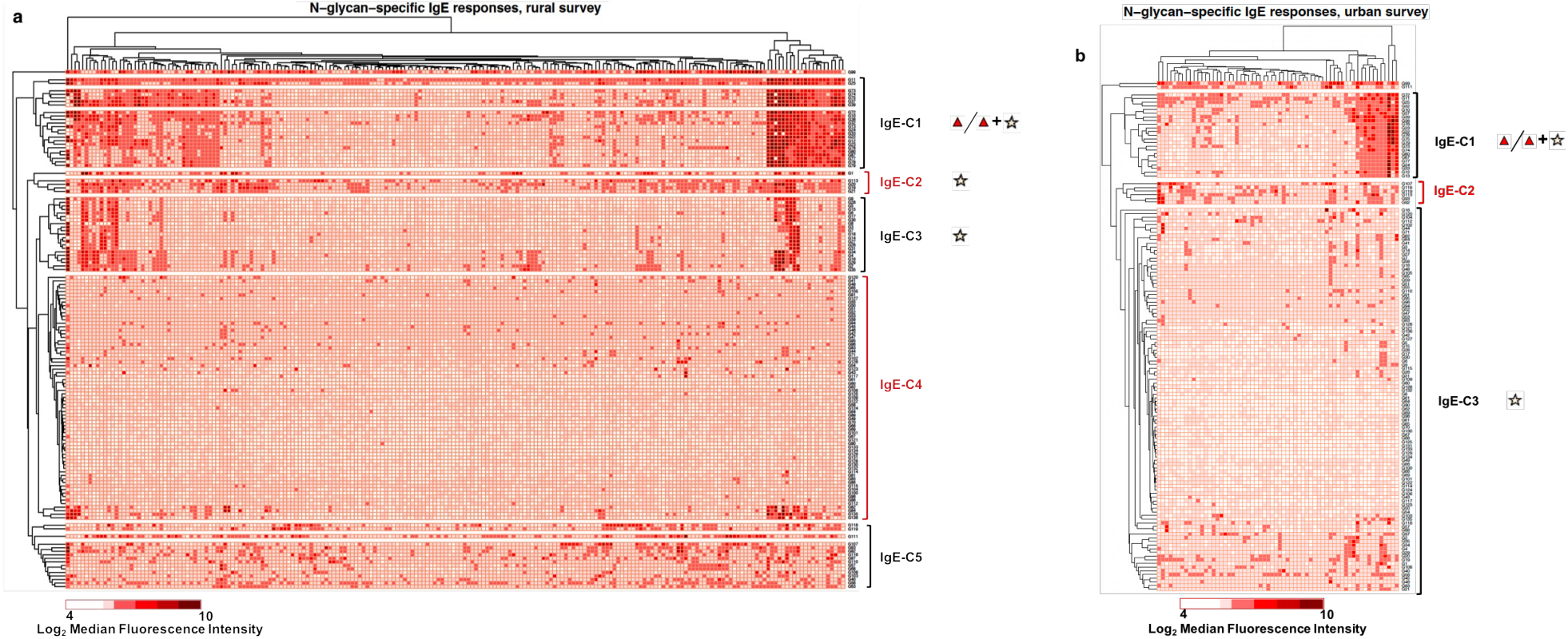


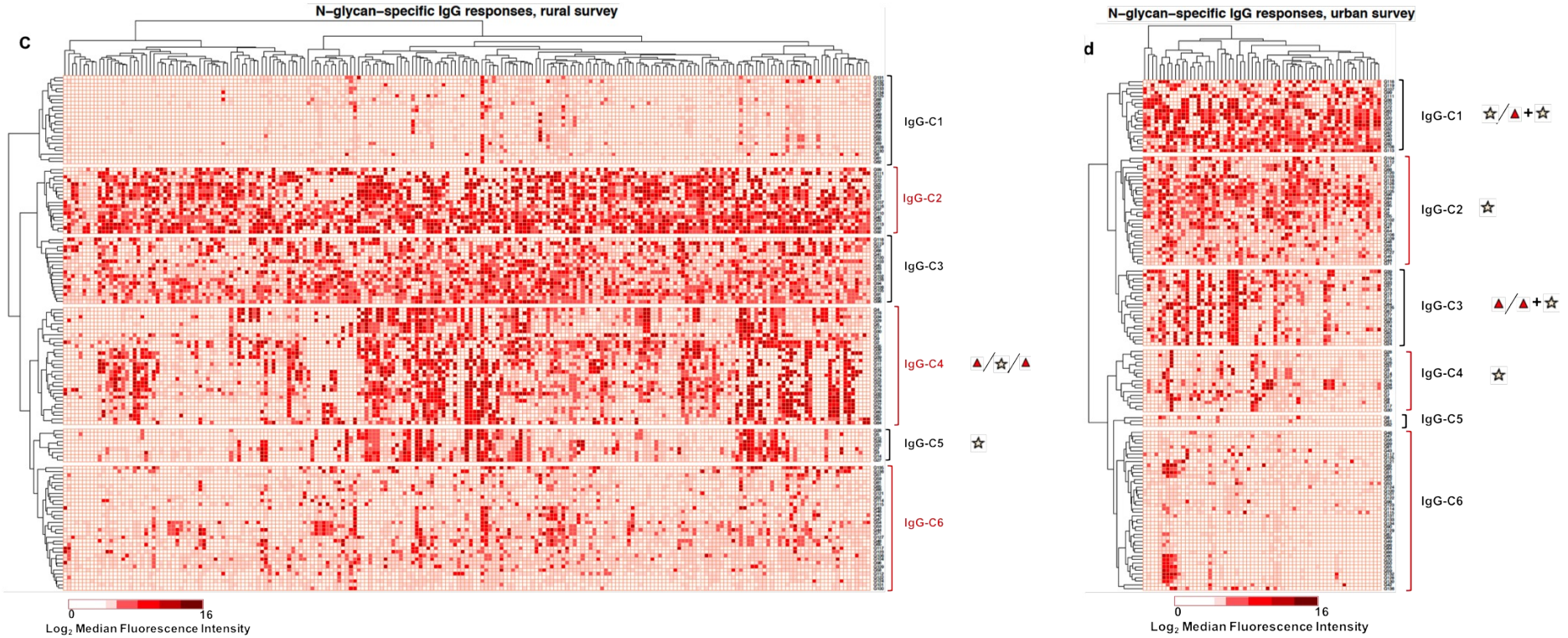
URBAN



Plasma from *S. mansoni* infected and uninfected rural and urban individuals were assessed for IgE and IgG reactivity to N-glycan structural variants. Box-and-whisker plots show background-subtracted and log_2-transformed median fluorescence intensities (MFI) representing IgE (a, b, c, g, h) and IgG (d, e, f, i, j) reactivity to the conserved, non-modified N-glycan core structure ($Man_3GlcNAc_2$, **G42**) and to N-glycan core structures carrying LDN (**G63**), LDNF (**G90**) and Lewis X (**G89**) antennae.
Mann-Whitney (IgE responses) and unpaired t test (IgG responses) were conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations, to assess differences between infected and uninfected individuals. The Kruskal-Wallis (IgE responses) and one-way ANOVA test (IgG responses) were also conducted using the permutation approach to assess differences along the infection intensity gradient (b and e) in the rural survey.
*p<0.05; **p<0.01; ***p<0.001.
Sm: *S. mansoni* infection determined by detection of eggs in a single stool sample by Kato-Katz and/or PCR (rural infected n=84, uninfected n=113; urban infected n=6, uninfected n=42).
CCA: *S. mansoni* infection determined by a positive urine circulating cathodic antigen (CCA) result (rural infected n=118, uninfected n=81; urban infected n=21, uninfected n=37).

Figure S4. Hierarchical cluster analysis of anti-glycan IgE and IgG responses





Hierarchical cluster analysis (complete linkage using Euclidean distance) was conducted using the 'pheatmap' package in R. Background-subtracted and log_2-transformed IgE and IgG median fluorescence intensities are shown on the y-axes, within clusters. The dominant core modifications on the glycans in these clusters are also shown. X-axes represent individual participants. **a)** Five major clusters of IgE responses were identified in the rural survey: cluster 1 (IgE-C1) comprised glycans carrying either only the core α3Fuc or both the core α3Fuc and β2Xyl, IgE-C2 and IgE-C3 comprised β-1,2-xylosylated glycans only, IgE-C4 and IgE-C5 comprised non-xylosylated and non-fucosylated glycans. **b)** Three major IgE clusters identified in the urban survey: IgE-C1 comprised glycans with core α3Fuc or both core α3Fuc and β2Xyl, IgE-C2 comprised non-xylosylated and non-fucosylated glycans, IgE-C3 comprised a mixture of β-1,2-xylosylated, non-xylosylated and non-fucosylated glycans. **c)** Six major IgG clusters identified in the rural survey: IgG-C1, IgG-C2, IgG-C3 and IgG-C6 were dominated by non-xylosylated and non-fucosylated glycans, IgG-C4 comprised glycans with either core α3Fuc or β2Xyl or both, IgG-C5 comprised β-1,2-xylosylated glycans only. **d)** Six major IgG clusters in the urban survey: IgG-C1 comprised glycans with either only core β2Xyl or both the core α3Fuc and β2Xyl, IgG-C2 a mixture of β-1,2-xylosylated, non-xylosylated and non-fucosylated glycans, IgG-C3 comprised glycans carrying either only core α3Fuc or both core α3Fuc and β2Xyl. IgE-C5 and IgE-C6 comprised non-xylosylated and non-fucosylated glycans.

Table S2. Global test p-values for associations between anti-glycan antibody response clusters and *S. mansoni* infection

RURAL	IgE-C1 (▲ / ▲ + ☆)	IgE-C2 (☆)	IgE-C3 (☆)	IgE-C4	IgE-C5	
Sm (KK)	0.284	**<0.001**	**0.019**	**0.036**	0.533	
Sm (PCR)	0.795	**0.039**	0.353	0.431	0.556	
CCA	0.872	0.681	0.617	0.928	0.992	
Infection intensity (KK)	0.385	**<0.001**	**0.003**	**0.032**	0.850	
URBAN	IgE-C1 (▲ / ▲ + ☆)	IgE-C2	IgE-C3 (☆)			
Sm (KK)	0.371	0.826	**0.002**			
Sm (PCR)	0.314	0.604	0.134			
CCA	0.733	0.652	0.421			
Infection intensity (KK)	0.904	0.925	**<0.001**			
RURAL	IgG-C1	IgG-C2	IgG-C3	IgG-C4 (▲ / ☆ / ▲)	IgG-C5 (☆)	IgG-C6
Sm (KK)	0.319	**0.028**	0.150	0.064	0.056	0.451
Sm (PCR)	0.701	0.373	0.241	0.180	0.090	0.539
CCA	0.175	0.934	0.939	0.695	0.678	0.394
Infection intensity (KK)	0.117	0.054	0.196	**0.019**	**0.024**	0.285
URBAN	IgG-C1 (☆ / ▲ + ☆)	IgG-C2 (☆)	IgG-C3 (▲ / ▲ + ☆)	IgG-C4 (☆)	IgG-C5	IgG-C6
Sm (KK)	0.834	0.077	0.371	0.066	**0.031**	0.237
Sm (PCR)	0.498	0.252	0.180	0.103	0.569	0.408
CCA	0.775	0.875	0.945	0.253	0.596	0.679
Infection intensity (KK)	0.807	0.167	0.828	0.106	**0.022**	0.368

Table shows age and sex-adjusted global test p-values obtained from a linear regression analysis in R using the 'Globaltest' package. The global test, described by Goeman et al., 2006[1], was used here to assess associations between anti-glycan IgE and IgG clusters (from hierarchical cluster analysis) and *S. mansoni* infection. All statistically significant associations shown in this table are positive, except for the association between IgG-C5 (Urban survey) and *Sm* (KK) and infection intensity.

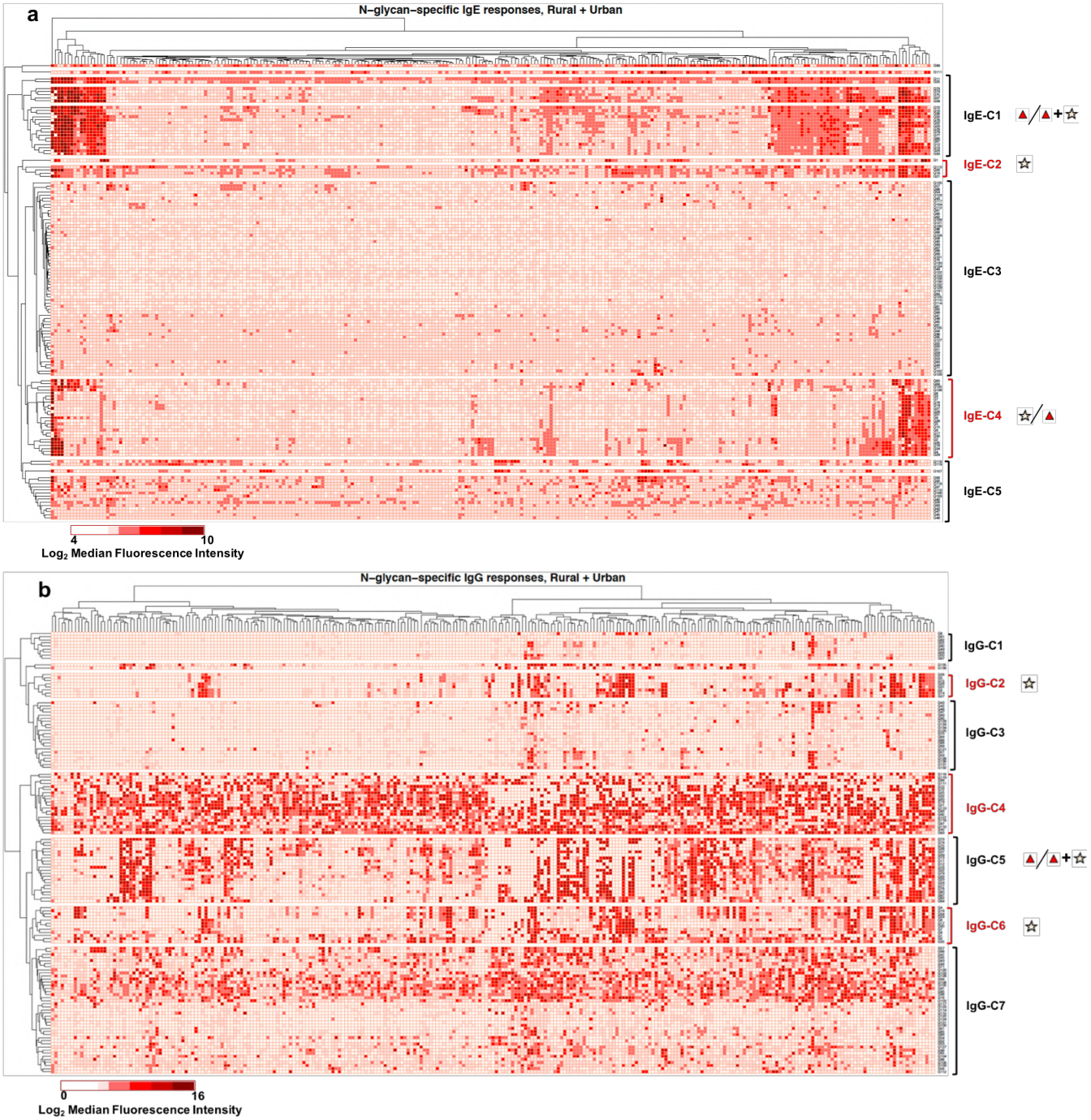
KK: Kato-Katz; **PCR**: Polymerase Chain Reaction; **CCA**: Circulating Cathodic Antigen; *Sm*: Schistosoma mansoni

Table S3. Global test p-values for associations between anti-glycan antibody response clusters and *Schistosoma*-specific antibodies

RURAL	IgE-C1 (▲ / ▲ + ☆)	IgE-C2 (☆)	IgE-C3 (☆)	IgE-C4	IgE-C5	
SWA-specific IgE	**<0.001**	**0.003**	**0.001**	**0.008**	0.073	
SEA-specific IgE	**<0.001**	**0.008**	**<0.001**	0.523	**0.031**	
URBAN	IgE-C1 (▲ / ▲ + ☆)	IgE-C2	IgE-C3 (☆)			
SWA-specific IgE	**<0.001**	0.255	**<0.001**			
SEA-specific IgE	**0.021**	0.372	**0.022**			
RURAL	IgG-C1	IgG-C2	IgG-C3	IgG-C4 (▲ / ☆ / ▲)	IgG-C5 (☆)	IgG-C6
SWA-specific IgG	0.079	**<0.001**	**0.005**	**<0.001**	**<0.001**	0.077
SEA-specific IgG	0.453	**<0.001**	**0.012**	**<0.001**	**0.003**	0.221
URBAN	IgG-C1 (☆ / ▲ + ☆)	IgG-C2 (☆)	IgG-C3 (▲ / ▲ + ☆)	IgG-C4 (☆)	IgG-C5	IgG-C6
SWA-specific IgG	0.219	0.518	**0.034**	**0.045**	0.872	0.357
SEA-specific IgG	0.063	0.303	**0.016**	0.075	0.918	0.434

Table shows age and sex-adjusted global test p-values obtained from a linear regression analysis in R using the 'Globaltest' package. The global test (Goeman et al., 2006[1]) was used to assess associations between anti-glycan IgE and IgG clusters (defined by hierarchical clustering analysis) and *Schistosoma* adult worm (SWA) and egg (SEA)-specific IgE and IgG. All statistically significant associations shown in this table are positive.

Figure S5. Hierarchical cluster analysis of anti-glycan antibody responses in individuals from both rural and urban settings



Hierarchical cluster analysis (complete linkage using Euclidean distance) of N-glycan-specific IgE and IgG responses (y-axis). X-axes represent individual participants.

Table S4. Global test p-values for associations between anti-glycan antibody response clusters and survey setting

IgE-C1 (▲/ ▲ + ☆)	IgE-C2 (☆)	IgE-C3	IgE-C4 (▲ / ☆)	IgE-C5		
0.095*	**0.024***	**0.006#**	0.094*	0.146*		
IgG-C1	IgG-C2 (☆)	IgG-C3	IgG-C4	IgG-C5 (▲/ ▲ + ☆)	IgG-C6 (☆)	IgG-C7
0.245#	0.508*	0.179*	0.222*	0.123*	0.223*	**0.009#**

Table shows age and sex-adjusted global test p-values obtained from a linear regression analysis in R using the 'Globaltest' package. The global test (Goeman et al., 2006[1]) was used to assess associations between anti-glycan IgE and IgG clusters (defined by hierarchical clustering analysis) and survey setting.

*Positive association between antibody response cluster and rural setting

#Positive association between antibody response cluster and urban setting

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1 Goeman, J. J., Van De Geer, S. A. & Van Houwelingen, H. C. Testing against a high dimensional alternative. *Journal of the Royal Statistical Society: Series B* **68**, 477-493 (2006).

CHAPTER 7. HELMINTH CARBOHYDRATE-SPECIFIC IgE AND ITS ROLE IN THE EPIDEMIOLOGY OF ALLERGY IN UGANDA

7.1 Preamble

Findings presented in this chapter are from analyses of data obtained from the rural and the urban survey, and from the asthma case-control study. These studies are described in detail in Chapter 3. The present chapter assesses the role of cross-reactive carbohydrate determinant (CCD)-specific IgE in the epidemiology of allergic sensitisation and disease (asthma) in Uganda (**thesis objective 4**). Results are presented in Research paper 4 (below), titled "Schistosomiasis-associated carbohydrate-specific IgE and the epidemiology of allergy: studies from Uganda". This manuscript has been submitted for publication as an original article in the *Journal of Allergy and Clinical Immunology.*

As shown in Chapter 6, the rural environment and *S. mansoni* infection and intensity were strongly associated with antibody reactivity to core β-1,2-xylose and α-1,3-fucose substituted N-glycans. These N-glycan core substitutions are integral constituents of classical CCDs, which are some of the commonest sources of cross-reactivity between *S. mansoni* and allergens. This chapter explores impact of CCD-specific IgE on allergic sensitisation and disease (asthma), hypothesising that CCD-specific IgE obscures true allergic sensitisation, and may be involved in protection against clinical allergy.

7.2 Research Paper 4: Schistosomiasis-associated carbohydrate-specific IgE and the epidemiology of allergy: studies from Uganda

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Student	GYAVIIRA NKURUNUNGI
Principal Supervisor	ALISON ELLIOTT
Thesis Title	HELMINTH-ALLERGY ASSOCIATIONS IN RURAL AND URBAN UGANDA: INSIGHTS FROM ANTIBODY STUDIES

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
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Where is the work intended to be published?	JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY
Please list the paper's authors in the intended authorship order:	Gyaviira Nkurununngi, Harriet Mpairwe, Serge Versteeg, Angela van Diepen, Jacent Nassuuna, Joyce Kabagenyi, Irene Nambuya, Richard E Sanya, Margaret Nampijja, Sonia Serna, Niels-Christian Reichardt, Cornelis H Hokke, Emily L Webb, Ronald van Ree, Maria Yazdanbakhsh, Alison M Elliott
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I contributed to the conception and experimental design of the study. I conducted the immunological experiments. I analysed the data and wrote the manuscript.

Student Signature: [redacted] Date: 15/02/2019

Supervisor Signature: [redacted] Date: 15/2/2019

Schistosomiasis-associated carbohydrate-specific IgE and the epidemiology of allergy: studies from Uganda

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Disclosure of potential conflict of interest

The authors declare that they have no conflicts of interest.

SUMMARY

Background

Cross-reactive carbohydrate determinants (CCDs) on schistosome and allergen glycoproteins may influence allergy epidemiology in the tropics.

Objective

We explored the impact of *Schistosoma mansoni (Sm)* exposure and rural-urban environment on anti-CCD IgE, and its relevance to allergic sensitisation and asthma.

Methods

Among Ugandan residents of rural *Sm*-endemic islands (n=780), proximate urban communities with lower helminth exposure (n=345), and asthmatic (n=200) and non-asthmatic schoolchildren (n=200), we measured IgE against house dust mite (HDM), cockroach and peanut extracts by ImmunoCAP®. In subsets, we measured IgE to allergen components by ISAC® microarray, and to core β-1,2-xylose and/or α-1,3-fucose substituted N-glycans ("classical" CCD targets for IgE) by glycan microarray.

Results

IgE sensitisation to crude allergen extracts was highly prevalent (55% in rural, 43% in urban participants; 73% in asthmatics, 54% in controls). By contrast, IgE sensitisation to established major allergenic components from HDM, cockroach and peanut was low amongst rural, urban and non-asthmatic participants (0%-12%, versus 18%-36% in asthmatics). Instead, up to 40% of all participants recognised insect venom and CCD-bearing components. Reactivity to classical CCD epitopes was positively associated with *Sm* infection, rural environment and allergen extract-specific IgE, but not with sensitisation to major allergenic components. CCD-specific IgE was not associated with skin reactivity; however, reactivity specifically to core α-1,3-fucosylated N-glycans was lower among asthmatics.

Conclusion

Reactivity to *Sm* CCDs obscures IgE sensitisation to established major allergenic components. The inverse association between reactivity to α-1,3-fucosylated CCD epitopes and asthma merits further investigation for a possible protective role in development of allergic disease.

KEY WORDS

Cross-reactive carbohydrate determinant (CCD), helminth, *Schistosoma mansoni*, allergy, asthma, IgE sensitisation, ISAC, ImmunoCAP, microarray, β-1,2-xylose, α-1,3-fucose

KEY MESSAGES

1. In Uganda, sensitisation to allergen extracts does not reflect sensitisation to their established major allergenic components, but rather to other, cross-reactive environmental antigens, particularly CCDs expressed by some helminths.
2. Reactivity to α-1,3-fucose, a "classical" CCD target for IgE, is inversely associated with asthma.

CAPSULE SUMMARY

In Uganda, IgE responses to classical CCD glycans are not merely a smokescreen that obscures readouts for atopy assessment: IgE to core α-1,3-fucosylated CCD epitopes associates inversely with clinical allergy, through yet-to-be-defined mechanisms.

ABBREVIATIONS USED

LICs: low-income countries; **CCD**: cross-reactive carbohydrate determinant; ***Sm***: *Schistosoma mansoni*; **LaVIISWA**: the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases; **ISAAC**: International Study on Allergy and Asthma in Children; **SPT**: skin prick test; **SWA**: *Schistosoma* adult worm antigen; **SEA**: *Schistosoma* egg antigen; **PCR**: polymerase chain reaction; **CCA**: circulating cathodic

antigen; **ISAC**: Immuno Solid-phase Allergen Chip; **ISU**: ISAC standardized units; **HDM**: house dust mite; **PCA**: principal component analysis

INTRODUCTION

There are important similarities in host immune responses to helminths and allergens, and in their molecular targets.[1] Helminths elicit strong, complex immunoregulatory networks to block allergy-like responses induced during infection, primarily to avert their own destruction and elimination.[2] Spill-over effects of this immunoregulation include inhibition of allergic effector responses,[3] perhaps contributing to lower prevalence of clinical allergies in low-income countries [LICs] (compared to high-income countries) and rural (compared to urban) settings.[4] However, studies in human populations imply diverse and complex relationships between helminth infections and allergy-related outcomes. For example, in rural *Schistosoma mansoni* (*Sm*)-endemic Ugandan fishing communities, we found that low prevalence of clinical allergies was paralleled by high levels of specific IgE against extracts from common allergen sources and positive helminth-atopy associations.[5]

Mechanistic studies assessing the impact of protein cross-reactivity between helminths and allergens have provided important insights into helminth-allergy associations.[6] For example, the *Onchocerca volvulus* tropomyosin (structurally homologous to dust mite tropomyosins) induces basophil histamine release[7] and some *Sm* venom allergen-like (SmVAL) proteins (with orthologues in wasps) and tegumental allergen-like (SmTAL) proteins show allergenic activity.[8,9]

The importance of carbohydrate cross reactivity in helminth-allergy associations is less understood. Some of the commonest sources of cross-reactivity between allergens and some helminths are specific types of asparagine (N) linked glycan modifications found on plant, insect and some helminth proteins (termed cross-reactive carbohydrate determinants, CCDs). The N-glycan trimannosyl-chitobiose core ($Man_3GlcNAc_2$) is conserved in eukaryotes. “Classical” CCD N-glycans expressed by invertebrate and plant proteins,[10,11] and on antigenic surfaces and in secretomes of schistosome eggs [12] and some nematodes,[13-16] carry additional, non-mammalian, IgE-binding motifs: 1) β-

(1,2) xylose linked to the first mannose of the trimannosyl component and/or 2) α-(1,3) fucose linked to the asparagine-linked N-acetylglucosamine (GlcNAc) of the glycan core.[17,18] Glycoproteins carrying such N-glycans are potent immune determinants,[10,19-22] inducing strong Th2-type responses,[23] and comprising epitopes for antibodies,[24-27] including IgE.[28-30] However, the relevance of these glycan motifs in allergy is unclear. Barring observations from a few studies,[31-33] anti-CCD IgE has poor biological function: individuals with specific IgE to CCDs lack skin and oral reactivity to the same molecules.[10,29,34] Therefore, in helminth-endemic LICs, elevated, yet clinically irrelevant allergen extract-specific IgE levels may, to an important extent, be attributed to CCDs.[35] Among Ghanaian schoolchildren, *Schistosoma haematobium* infection was associated with peanut-IgE-sensitisation, which was in turn strongly associated with anti-CCD IgE, but not clinical peanut allergy.[36] The separation of anti-CCD IgE from clinical allergy symptoms, coupled with abundance of immunogenic CCDs on some schistosome antigens, led us to hypothesise that enhanced anti-CCD IgE responses during chronic *Sm* infection might dominate over allergen protein-specific IgE, resulting in reduced allergic effector responses.

We conducted three studies in varied Ugandan settings to obtain a comparative assessment of allergy-related disease phenotypes, prevalence and risk factors in rural[37] and urban[38] settings, and among asthmatic children and controls. These studies provide an unprecedented opportunity to assess the impact of rural-urban environment and *Sm* exposure on anti-CCD IgE profiles and the relevance of these profiles to epidemiological trends of allergy in tropical LICs.

METHODS

Study design and population

The three studies comprised rural and urban cross-sectional surveys on allergy outcomes and a case-control study on asthma risk factors among schoolchildren in Uganda.

The rural survey (September 2015 – August 2016) was conducted in *Sm*-endemic fishing villages of Koome islands, Lake Victoria, Uganda. It was the three-year outcome survey of the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA; ISRCTN47196031[39]), a cluster-randomised trial of community-wide standard versus intensive anthelminthic treatment.[5,39] The urban survey (September 2016 – September 2017) was conducted in Entebbe municipality, a lower helminth exposure, urban setting[38] situated on northern shores of Lake Victoria.

The asthma study (May 2015 – July 2017) enrolled children with asthma and controls from schools in Entebbe and surrounding areas. Asthma was defined as history of wheeze in the last year, using the International Study on Allergy and Asthma in Children (ISAAC) questionnaire.[40] Children in the same class as cases, with no history of wheezing, were eligible as controls. A Stata program (College Station, Texas, USA) was used to randomly select participants from each class register such that the number of controls was twice the number of cases.

Recent wheeze was also assessed in the rural and urban surveys using ISAAC questionnaires. Other procedures were identical in all three studies, including assessment of skin prick test (SPT) reactivity to *Dermatophagoides* mix, *Blomia tropicalis* and *Blattella germanica* (ALK-Abelló; supplied by Laboratory Specialities [Pty] Ltd., South Africa), visible flexural dermatitis (evaluated following Williams' on-line manual[41]), and questionnaire-determined recent rhinitis and urticarial rash.

Parasitological examinations

Infection with intestinal helminths was investigated using the Kato-Katz technique[42] conducted on one stool sample per participant (two slides, read by different technicians). In the urban and rural surveys, stool was further examined for *Sm, Necator americanus* and *Strongyloides stercoralis* infections using PCR[43,44], and urine for *Sm* circulating cathodic antigen (CCA, Rapid Medical Diagnostics, South Africa).

Measurement of allergen- and glycan-specific IgE

Allergen- and glycan-specific IgE measurements were conducted in a subset of samples per study (Figure 1), randomly selected using Stata software. Sample size considerations are detailed in the Online Repository.

Crude house dust mite (*D. pteronyssinus,* HDM), peanut (*Arachis hypogaea*), and German cockroach (*B. germanica*) extract-specific plasma IgE levels were measured using the ImmunoCAP® specific IgE test [Thermo Fisher Scientific] (hereinafter 'ImmunoCAP'), with ≥0.35kU/L defining allergic sensitisation.[45]

The ImmunoCAP® ISAC (Immuno Solid-phase Allergen Chip) microarray (Thermo Fisher Scientific)[46,47] was used to measure IgE to 112 allergen components from 51 sources (Table S1, Online Repository). The array comprises 67 recombinant components (produced in *E. coli*, hence non-glycosylated[48]); and 45 components purified from natural extracts. Previous work has shown that the glycan epitope from bromelain (nMUXF3) and glycoproteins from pollen (nPhl p 4, nCyn d 1, nPla a 2, nCry j 1, nCup a 1) and food (nJug r 2), are recognised by anti-CCD IgE, while other natural, possibly glycosylated allergens on the ISAC are not.[49] Therefore, herein, 'CCD-bearing components' denotes ISAC components confirmed to carry core β-1,2-xylose and/or α-1,3-fucose substituted N-glycans. The binding assay is described in Online Repository. Participants with an ISAC Standardised Unit (ISU) measurement of ≥0.3 were considered sensitised to that allergen component.[46,50] Measurements were also reported as detectable versus undetectable (lower detection limit: 0.06 ISU).

A non-commercial microarray of 135 chemo-enzymatically synthesised glycans with and without β-1,2-xylosylation and/or α-1,3-fucosylation (Figure S1) was employed to measure plasma anti-glycan IgE. Microarray construction procedures have been published.[51,52] The IgE binding assay and the microarray image processing procedures were adapted from previous studies,[27,53-55] and are detailed in Online Repository. Median fluorescence intensities (MFIs) reported herein represent anti-glycan IgE concentrations, because fluorescence-labelled anti-human IgE was used to detect plasma IgE bound to individual glycans on the array.

Samples were also assessed for *Schistosoma* egg [SEA]- and adult worm [SWA] antigen-specific IgE, IgG and IgG4, by ELISA (described elsewhere[56]).

Statistical analysis

Statistical analyses were done in Stata 13.1, GraphPad Prism 7.0a (Fay Avenue, CA, USA) and R via the RStudio interface (version 1.1.383, Boston, USA). Initial analyses in the rural survey investigated the impact of trial intervention on IgE profiles using a cluster-level approach, as previously described.[37] Differences in characteristics between rural and urban survey participants and between asthmatic and non-asthmatic children were assessed using logistic or linear regression, allowing for the survey designs (clustering and weighting). Unadjusted analysis of differences in individual N-glycan- / allergen-specific IgE levels between rural and urban participants, and between asthmatics and controls was done using the Mann-Whitney U test, correcting for multiple testing using a Monte Carlo simulation approach[57] with 1000 permutations, to generate empirical p-values. For analyses comparing prevalences of ISAC-determined IgE sensitisation between these groups, chi-squared tests (or Fisher's exact test, for expected cell counts <5) were used.

Since anti-glycan IgE responses were strongly correlated, they were further analysed using principal component analysis (PCA). Participants from all studies were pooled and principal component (PC) scores generated. Unadjusted and age- and sex-adjusted

associations between PC scores and atopic sensitisation, asthma status, *Sm* infection and survey setting were assessed using linear regression.

Ethics statement

This work was approved by ethics committees of Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, and Uganda National Council for Science and Technology. Informed consent was obtained from all participants and/or their legal guardians. Assent was obtained from children ≥8 years.

RESULTS

Participants' characteristics

Data on ImmunoCAP-determined IgE sensitisation were available for 780, 345 and 400 participants of the rural survey, urban survey and asthma study, respectively (**Figure 1**). For the rural survey, we present data combined from both anthelminthic treatment arms, because there was no effect of trial arm on ImmunoCAP- or ISAC-determined IgE sensitisation, anti-glycan IgE reactivity (**Table S2**) or prevalence of *Sm* CCA positivity.[37]

Table 1 shows participants' characteristics. Rural participants were on average older than urban participants (p=0.001) and more likely to be male. SPT reactivity to *Dermatophagoides* mix (p=0.003) and *B. tropicalis* (p=0.025) was higher among urban participants, while total IgE (p<0.001) and allergen extract-specific IgE sensitisation (ImmunoCAP concentration ≥0.35 kU/L), particularly to cockroach (p<0.001), was higher among rural participants. Urticarial rash was more common in rural participants (p<0.001), while wheeze, rhinitis and dermatitis were rare in both settings. Rural, compared to urban participants, were more frequently infected with *Sm* (p<0.001), *T. trichiura* (p=0.002), hookworm (p=0.016) and *S. stercoralis* (p=0.001), and had higher median levels of SEA- and SWA-specific IgE, IgG and IgG_4 (p<0.001). Adjusting for age and sex did not impact these differences.

Asthmatics, compared to non-asthmatic controls, had higher prevalence of SPT reactivity, ImmunoCAP IgE sensitisation, dermatitis and rhinitis, and higher total IgE levels. Prevalence of helminth infections in the asthma study was low, but concentrations of SEA- and SWA-specific antibodies were moderate and similar between asthmatics and non-asthmatics.

Figure 1. *Selection of samples for the ImmunoCAP® test and the ISAC® and glycan microarray assays*

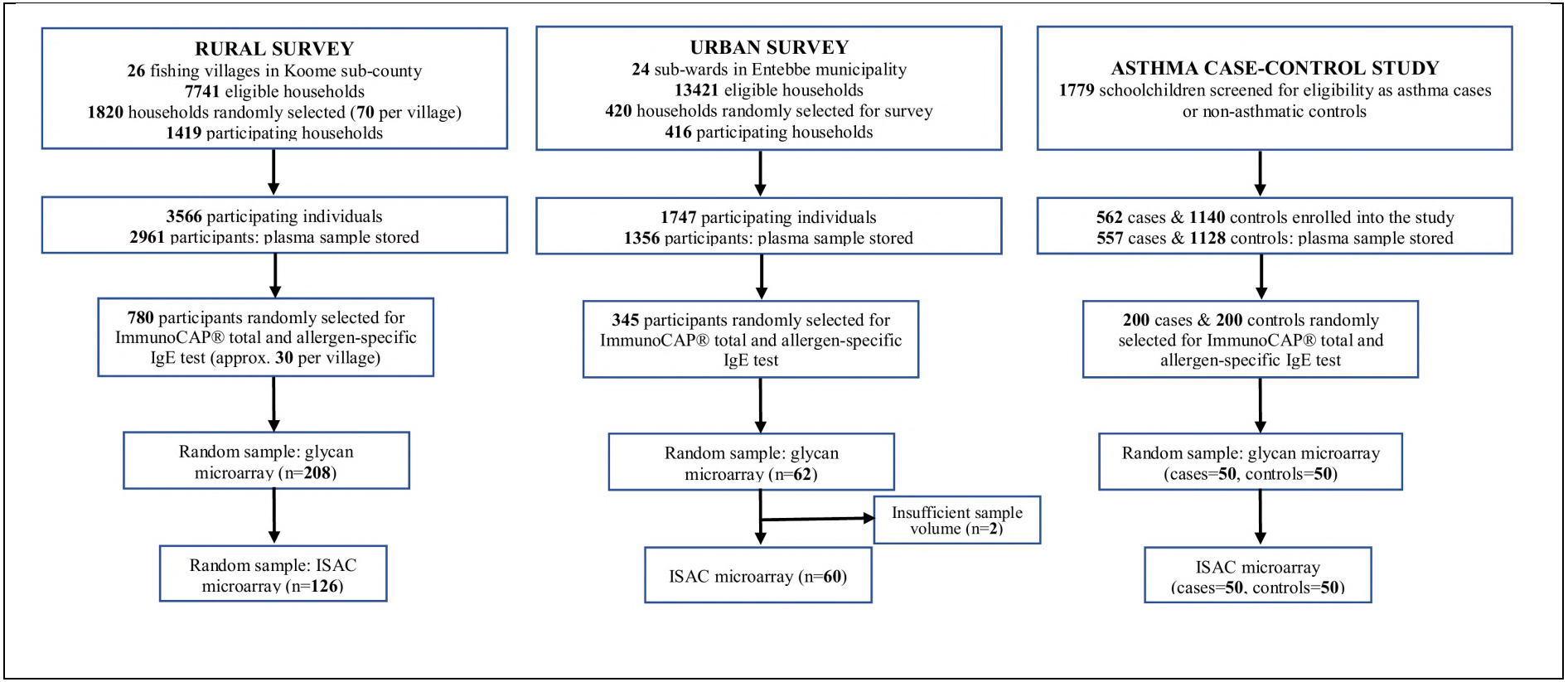


Table 1. *Characteristics of study participants*

Characteristics	Rural survey	Urban survey		Case-control study on asthma in schoolchildren n/N (%)		
	n/N (%)*	n/N (%)*	p¶	Non-asthmatics	Asthmatics	p
Socio-demographic						
Age in years, median (IQR)	**28 (21, 36)**	22 (10, 32)	**0.001**	10 (8, 13)	**11 (10, 14)**	**0.017**
Male sex	**377/780 (46.4)**	97/345 (28.1)	**<0.001**	94/200 (46.7)	92/200 (46.0)	0.841
Allergy-related outcomes						
Skin prick test reactivity						
Any	164/780 (21.3)	72/336 (21.4)	0.955	60/198 (30.3)	**105/198 (53.0)**	**<0.001**
Dermatophagoides mix	87/780 (10.0)	**61/336 (18.2)**	**0.003**	47/198 (23.7)	**91/198 (45.9)**	**<0.001**
Blomia tropicalis	54/780 (6.8)	**41/336 (12.2)**	**0.025**	45/198 (22.7)	**90/198 (45.4)**	**<0.001**
Blatella germanica	98/780 (13.9)	45/337 (13.4)	0.811	32/198 (16.2)	**48/198 (24.2)**	**0.046**
asIgE sensitisation (≥0.35 kU/L, ImmunoCAP)						
Any	**437/780 (55.1)**	148/345 (42.9)	**0.007**	108/200 (54.0)	**145/199 (72.9)**	**<0.001**
D. pteronyssinus	264/780 (33.2)	104/345 (30.1)	0.421	72/200 (36.0)	**117/200 (58.5)**	**<0.001**
Blatella germanica	**393/780 (49.8)**	118/345 (34.2)	**<0.001**	90/200 (45.0)	**112/199 (56.3)**	**0.025**
Arachis hypogaea	114/780 (14.9)	41/345 (11.9)	0.266	24/200 (12.0)	**39/200 (19.5)**	**0.041**
asIgE concentration (kU/L, ImmunoCAP), median (IQR)						
D. pteronyssinus	0.2 (0.0, 0.6)	0.1 (0.0, 0.7)	0.229	0.1 (0.0, 1.9)	**1.0 (0.1, 37.5)**	**<0.001**
Blatella germanica	0.4 (0.1, 1.5)	0.1 (0.0, 0.6)	0.949	0.3 (0.1, 1.1)	**0.6 (0.1, 2.9)**	**<0.001**
Arachis hypogaea	0.1 (0.0, 0.2)	0.0 (0.0, 0.1)	0.877	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)	0.101
Total IgE (kU/L, ImmunoCAP), median (IQR)	**672 (250, 1942)**	159 (57, 523)	**<0.001**	279 (98, 648)	**487 (115, 1248)**	**0.018**
Wheeze in last 12 months, age<5 years	1/58 (0.9)	2/37 (5.4)	0.188			
Wheeze in last 12 months, age≥5 years	24/716 (2.9)	5/272 (1.8)	0.308	0/200 (0.0)	200/200 (100.0)	
Visible flexural dermatitis	4/780 (0.5)	3/345 (0.9)	0.462	3/199 (1.5)	**13/198 (6.6)**	**0.019**
Rhinitis in last 12 months	34/774 (4.2)	11/309 (3.6)	0.700	11/199 (5.5)	**43/198 (21.7)**	**<0.001**
Urticaria in last 12 months	**98/773 (12.4)**	11/309 (3.6)	**<0.001**	5/199 (2.5)	6/198 (3.0)	0.754
Helminth infections						
S. mansoni (KK)	**187/679 (29.5)**	14/284 (4.9)	**<0.001**	8/194 (4.1)	12/184 (6.5)	0.302
S. mansoni intensity (KK)						
Uninfected	492/679 (70.5)	**270/284 (95.1)**		186/194 (95.9)	172/184 (93.5)	
Low	**94/679 (14.7)**	7/284 (2.5)		4/194 (2.1)	7/184 (3.8)	
Moderate	**53/679 (8.9)**	4/284 (1.4)		3/194 (1.5)	3/184 (1.6)	
Heavy	**40/679 (5.8)**	3/284 (1.1)	**<0.001**	1/194 (0.5)	2/184 (1.1)	0.328
S. mansoni (urine CCA)§	**590/724 (82.4)**	108/309 (34.9)	**<0.001**			
S. mansoni (PCR)§	**310/679 (47.5)**	43/282 (15.3)	**<0.001**			
A. lumbricoides (KK)	2/679 (0.2)	0/284 (0)		0/194 (0)	1/184 (0.5)	
T. trichiura (KK)	**44/679 (6.2)**	4/284 (1.4)	**0.002**	4/194 (2.1)	2/184 (1.1)	0.456
N. americanus (PCR)	**72/679 (9.9)**	12/282 (4.3)	**0.016**	3/194 (1.6)#	2/184 (1.1)#	0.697
S. stercoralis (PCR)§	**58/679 (7.5)**	4/282 (1.4)	**0.001**			
***Schistosoma*-specific antibody levels (µg/ml), median (IQR)**						
SEA-specific IgE	**4.6 (3.1, 6.6)**	2.6 (1.7, 4.4)	**<0.001**	2.4 (1.5, 4.0)	2.4 (1.7, 3.9)	0.566
SWA-specific IgE	**4.9 (3.0, 6.7)**	2.3 (1.5, 3.4)	**<0.001**	1.9 (1.3, 3.4)	2.0 (1.5, 3.3)	0.914
SEA-specific IgG_4	**282 (70, 839)**	27 (0, 90)	**<0.001**	27 (0, 77)	26 (0, 68)	0.255
SWA-specific IgG_4	**109 (52, 275)**	39 (18, 65)	**<0.001**	38 (22, 64)	39 (22, 60)	0.871
SEA-specific IgG	**1975 (1061, 3098)**	739 (599, 1476)	**<0.001**	736 (602, 1318)	693 (593, 1117)	0.686
SWA-specific IgG	**1499 (999, 2140)**	791 (612, 1200)	**<0.001**	825 (644, 1130)	737 (615, 1061)	0.495

Table shows characteristics for individuals with data on ImmunoCAP-determined IgE sensitisation. Probability values are shown for differences in characteristics between rural and urban survey participants and between asthmatic schoolchildren and non-asthmatic controls.

**Percentages were adjusted for survey design. Percentages / medians that were significantly higher in one group compared to the other (p≤0.05) are highlighted in bold. Adjusting for age and sex had little impact on these differences.*

¶*P values obtained from survey design-based logistic or linear regression.*

§*Information not collected in the asthma case-control study.*

#*Necator americanus infection detected by Kato-Katz in the asthma case-control study.*

***asIgE:** allergen-specific IgE; **KK**: Kato-Katz; **PCR**: Polymerase Chain Reaction; **CCA**: Circulating Cathodic Antigen; **IQR**: Interquartile range; **SEA**: Schistosoma egg antigen; **SWA**: Schistosoma adult worm antigen*

Overall responses to structures on the ISAC and N-glycan microarray

Figure 2 shows prevalence of sensitisation (IgE≥0.3 ISU) to allergen components on the ISAC microarray. Among rural participants, sensitisation to components on the array was dominated by reactivity to insect venom proteins (rPol d 5, rVes v 5, rApi m 1) and components bearing classical CCDs. Among urban participants and non-asthmatics, sensitisation patterns were more varied: HDM, venom, food and CCD-bearing components contributed most to sensitisation. However, sensitisation among asthmatics was dominated by reactivity to major recombinant and natural HDM allergens.

Figure 3 shows average MFIs for structures on the glycan microarray. Although the highest responses were raised predominantly against core β-1,2-xylose and/or α-1,3-fucose substituted N-glycans in all three studies, responses to a few other carbohydrate structural elements such as tri-mannose (G99), fucosylated GlcNAc (G111) and galactose-alpha-1,3-galactose (α-1,3-gal) [G112, G113] were also as high.

Figure 2. *Prevalence of sensitisation (IgE≥0.3 ISU) to allergen components on the ISAC microarray*

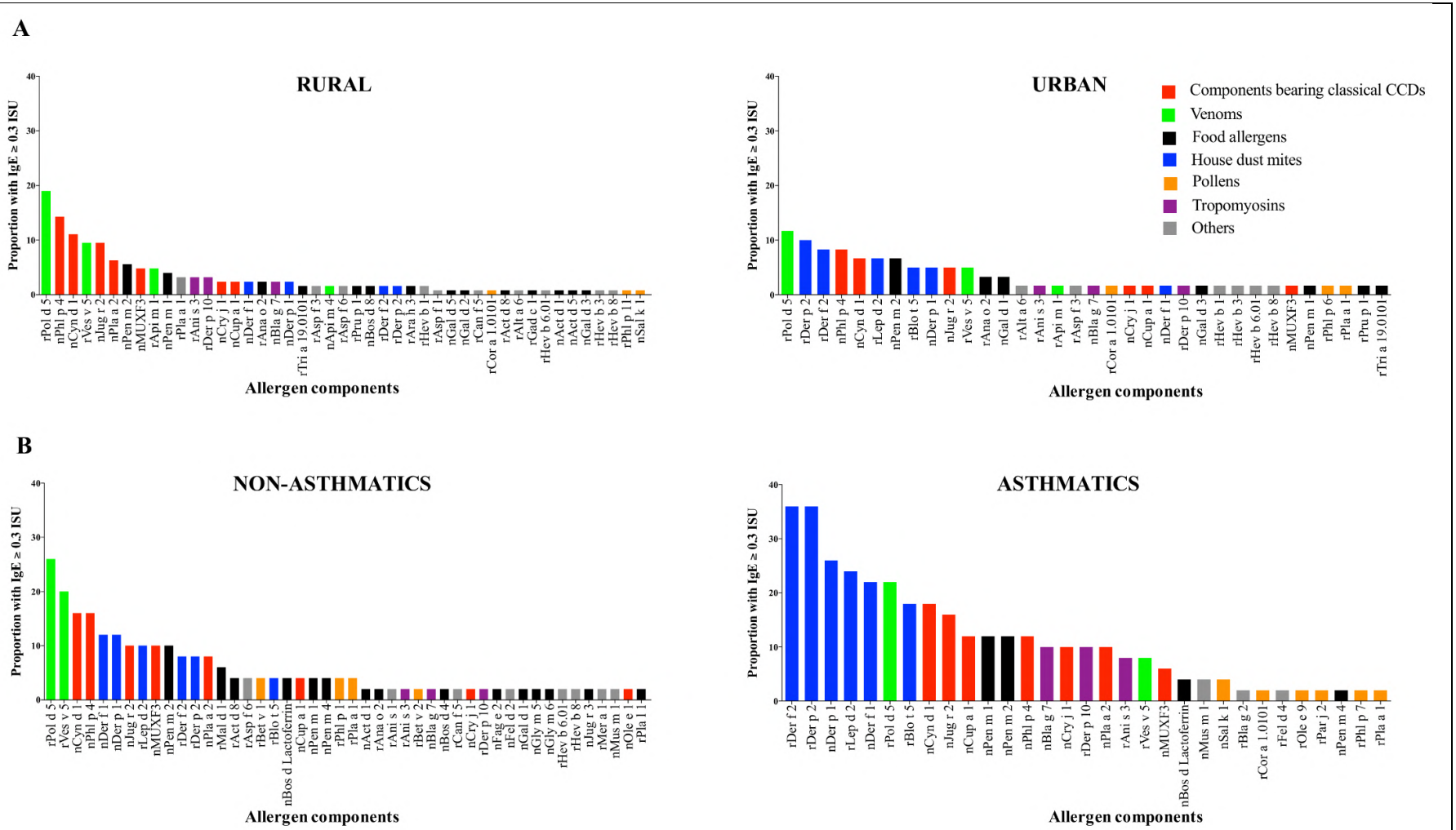
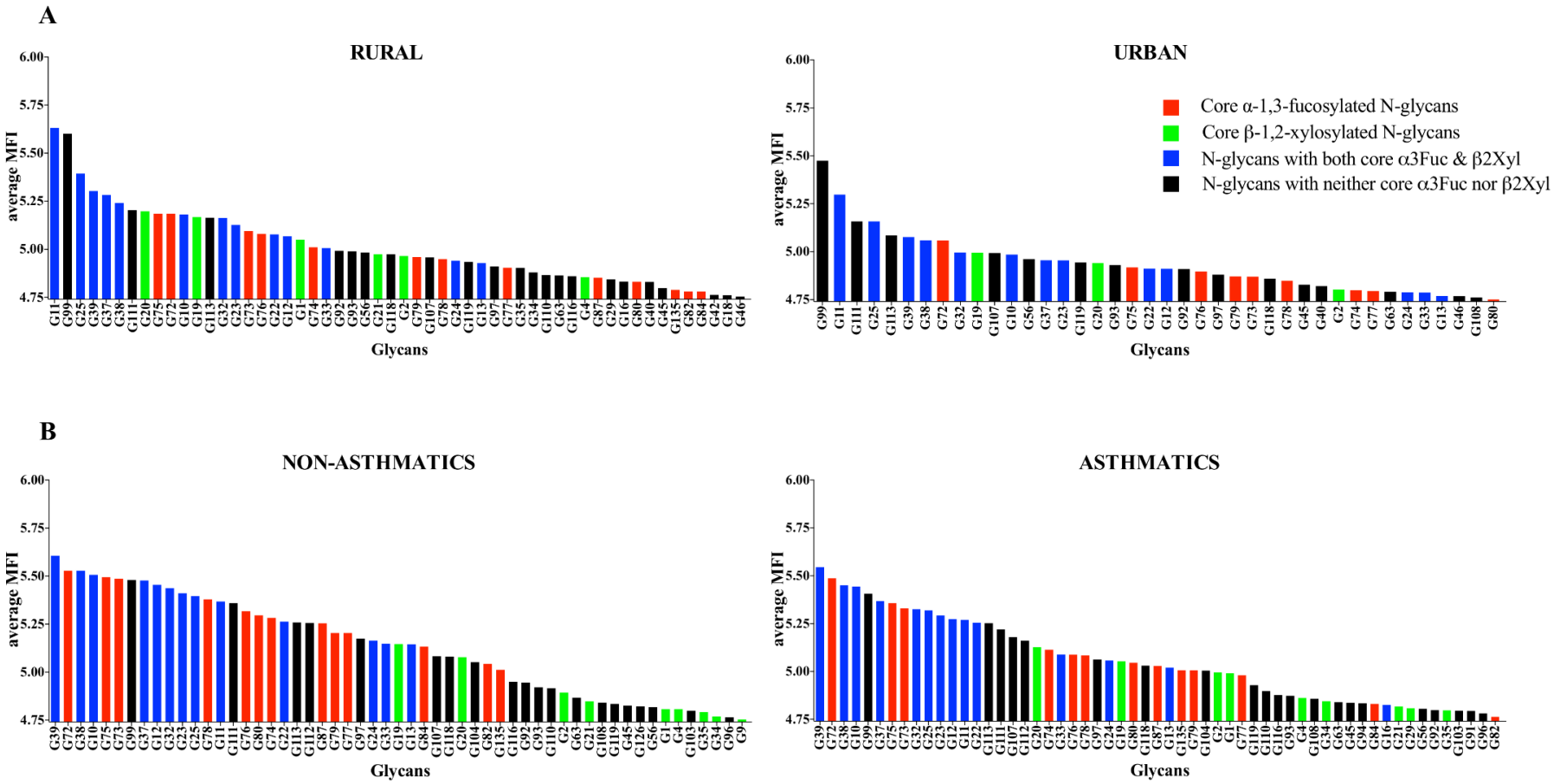


*Figure shows only allergen components to which IgE ≥ 0.3 ISU was raised. Natural and recombinant allergens are denoted by the prefixes (**n**) and (**r**), respectively, on the allergen name.* ***ISAC:*** *Immuno Solid-phase Allergen Chip;* ***ISU:*** *ISAC standardised units;* ***CCD:*** *Cross-reactive carbohydrate determinant*

Figure 3. *Responses to structures on the glycan microarray*



*Plasma from rural and the urban survey participants (**panel A**) and asthmatic and non-asthmatic schoolchildren (**panel B**) were assessed for IgE reactivity to glycan structural variants with and without α-1,3-fucosylation and β-1,2-xylosylation, on a microarray platform.*

Graphs show background-subtracted and log_2-transformed median fluorescence intensities (MFI). Only structures with an average MFI above 4.75 are shown. The entire collection of structures on the microarray is shown in supplementary Figure E1.

Sensitisation to allergen extracts does not reflect sensitisation to their commonly accepted major components

In all study settings, sensitisation (ImmunoCAP IgE ≥0.35 kU/L) to HDM, German cockroach and peanut extracts was high (**Table 1**). Fifty five percent and 43% of rural and urban participants and 73% and 54% of asthmatic schoolchildren and their controls, respectively, were sensitised to at least one of the three extracts. Skin reactivity to each extract was far lower than prevalence of IgE sensitisation to the same extracts.

However, sensitisation to both natural and recombinant forms of established major allergenic components of these extracts was very low among rural and urban survey participants, and among non-asthmatic schoolchildren (**Figure 4**): 0%-3% (rural), 0%-10% (urban) and 0%-12% (non-asthmatics) of tested participants were sensitised (IgE ≥0.30 ISU) to HDM, cockroach and peanut components on ISAC. The picture was strikingly different among asthmatics: 18%-36% were sensitised to HDM allergens (42% to at least one) and 0-10% to cockroach; but none to peanut components.

Reactivity to CCDs and venom proteins dominates ISAC-determined IgE profiles, and is strongly associated with the rural environment

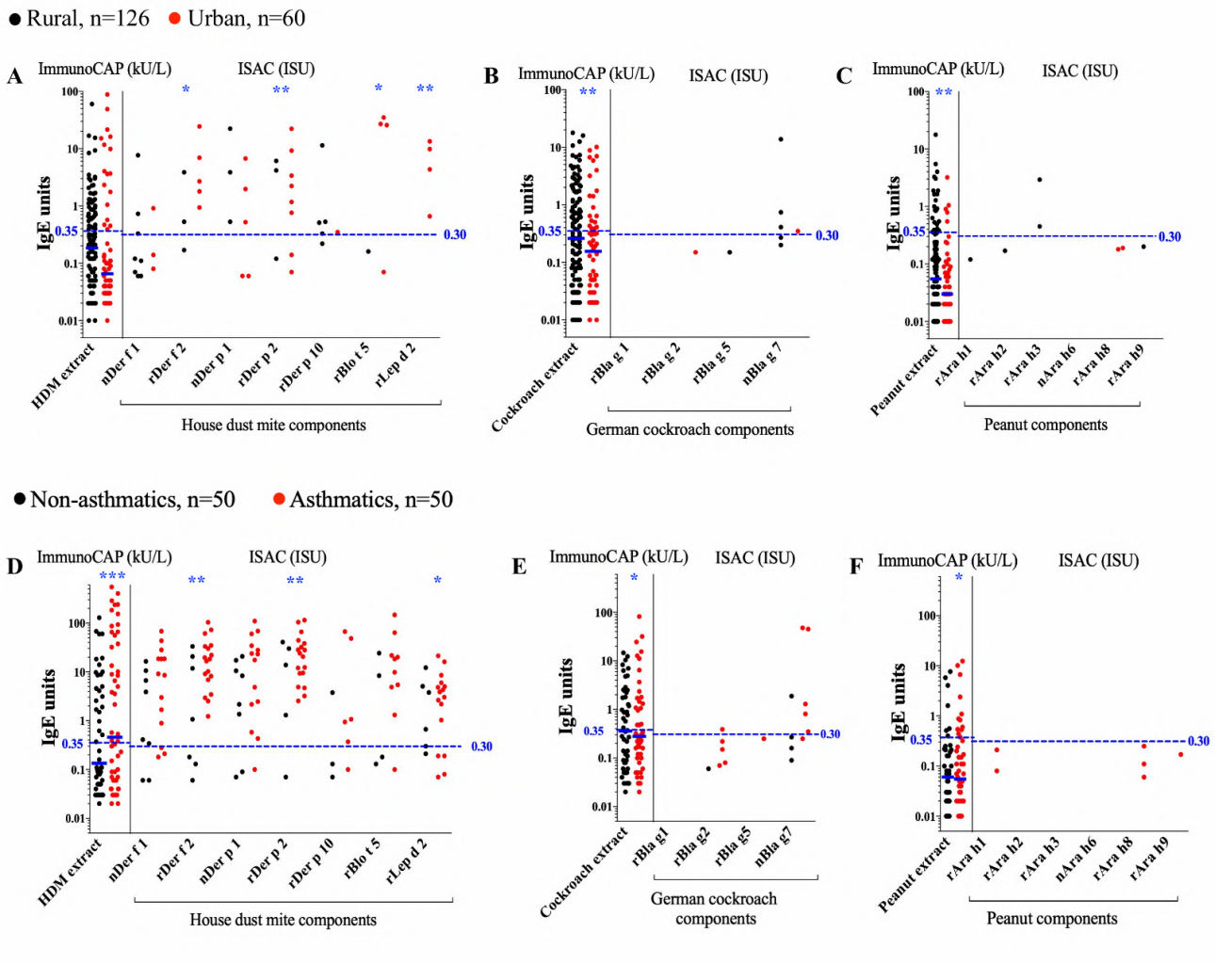
Despite the low prevalence of ISAC-determined IgE sensitisation to major natural and recombinant components of common allergens in our study settings,[58] many participants mounted responses to components bearing classical CCDs (**Figures 5A and 5C**) and wasp venom proteins (rVes v5, rPol d 5) (**Figures 5B and 5D**).

A higher proportion of rural, compared to urban participants, recognised CCDs and venom proteins (**Table S3** and **Figures 5A** and **5B**). However, this was statistically significant only for the CCDs nPhl p4 and nCry j 1 and the venoms rPol d 5 and rVes v 5. Conversely, a higher proportion of urban, compared to rural participants, recognised recombinant major allergens of HDM (**Table S3** and **Figure 4A**), the food components rApi g 1 and rMal d 1 and the cat allergen rFel d 4 (**Table S3**).

Although reactivity to HDM components was higher among asthmatics compared to

controls, reactivity to components bearing classical CCDs, venom proteins and most other allergen components on the array was generally similar between the two groups (**Figure 4** and **Table S3**).

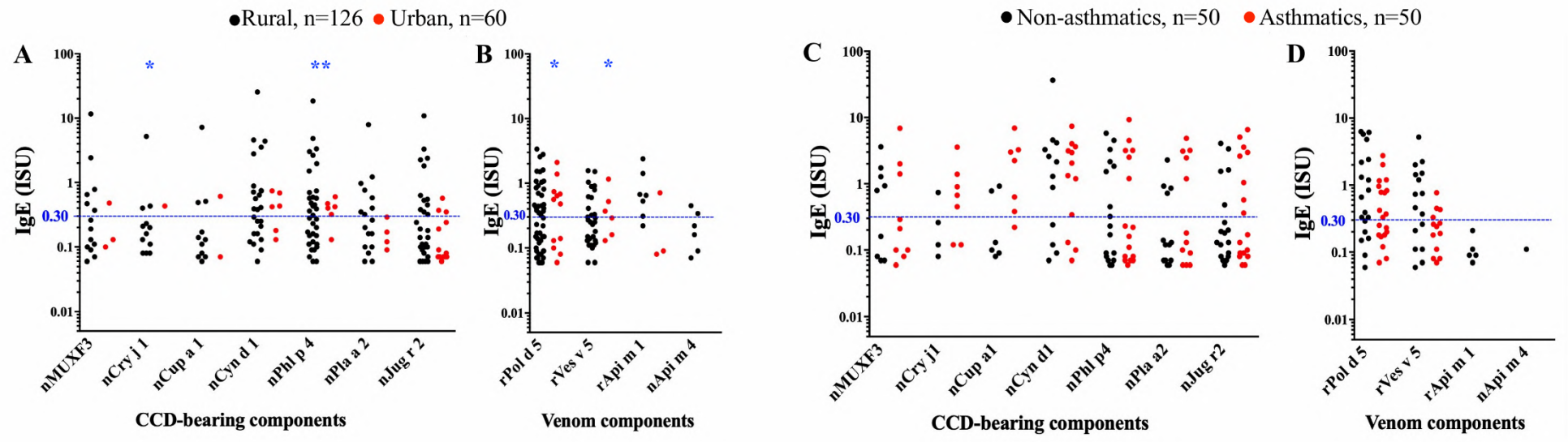
Figure 4. *IgE reactivity to allergen extracts and their major allergenic components*



*ImmunoCAP-determined IgE sensitisation to HDM, German cockroach and peanut extracts and ISAC-determined IgE sensitisation to the major allergenic components in these extracts. Natural and recombinant allergens on the ISAC array are denoted by the prefixes (**n**) and (**r**), respectively, on the allergen name. Cut-offs of 0.35 kU/L and 0.30 ISU are shown to define which ImmunoCAP and ISAC responses, respectively, are important for clinical diagnosis of allergic sensitisation. The Mann-Whitney U test was conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations (in order to adjust for multiple testing), to assess differences between rural and urban individuals (panels A, B and C), and between asthmatics and their controls (panels D, E and F): *p<0.05; **p<0.01; ***p<0.001.*

HDM: *house dust mite;* ***ISAC:*** *Immuno Solid-phase Allergen Chip;* ***ISU:*** *ISAC standardised units;* ***CCD:*** *cross-reactive carbohydrate determinant*

Figure 5. *IgE reactivity to venoms and CCD-bearing components on the ISAC array*



*Natural and recombinant allergens are denoted by the prefixes (**n**) and (**r**), respectively, on the allergen name. Cut-offs of 0.30 ISU are shown to define which ISAC-determined IgE responses are important for clinical diagnosis of allergic sensitisation.*

*The Mann-Whitney U test was conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations (in order to adjust for multiple testing), to assess differences between rural and urban individuals, and between asthmatics and their controls: *p<0.05; **p<0.01; ***p<0.001.*

***ISU**: ISAC standardised units; **CCD**: cross-reactive carbohydrate determinant*

Associations between helminth infections and ISAC-determined CCD- and venom-specific IgE

Schistosomes share common carbohydrate structures with some allergens.[18] A higher proportion of *Sm* infected compared to uninfected rural participants recognised CCDs and venom and fungal proteins on the ISAC array; however, this difference was statistically significant only for venom and fungal allergens (**Table S4**). Helminth prevalence was low in the urban survey and in the asthma study, hence associations with helminths were not examined there.

Reactivity to core β-1,2-xylose/α-1,3-fucose is positively associated with *Sm* infection and sensitisation to allergen extracts, but not to their established major components

Cross-reactive carbohydrate determinants expressed by several insect and plant glycoproteins, and by some nematodes and trematodes (such as schistosomes), are typified by presence of α-1,3-linked core fucose and β-1,2-linked core xylose motifs.[11,18] We combined the three studies and further explored associations between CCD-specific IgE and *Sm* infection and atopic sensitisation using microarray binding studies of core β-1,2-xylose and α-1,3-fucose substituted N-glycans.

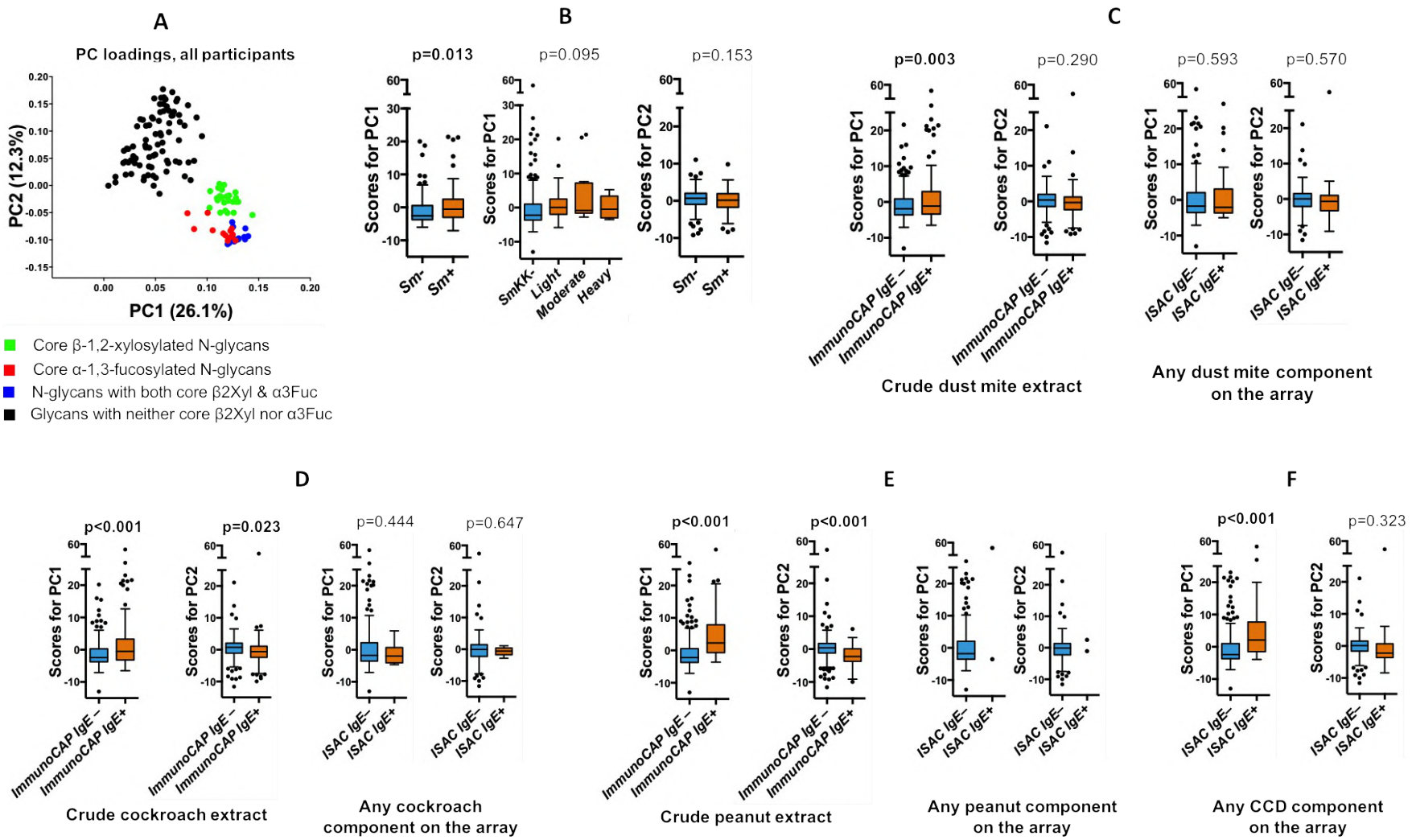
Responses to individual core substituted N-glycans were strongly correlated and were combined using PCA. **Figure 6A** shows scatterplots of PC1 and PC2 loadings (all participants). PC1 was characterised by responses to core β-1,2-xylose and/or α-1,3-fucose substituted N-glycans while PC2 was characterised by responses to non-xylosylated and non-fucosylated glycans. There were positive associations between PC1 scores and **1)** *Sm* infection (**Figure 6B)** [but not other helminths, data not shown], **2)** HDM, cockroach and peanut extract-sensitisation (on ImmunoCAP), **3)** CCD- and insect venom-sensitisation [on ISAC] (**Figures 6C-G**) and **4)** the rural environment (**Figure 6H**). However, no associations were observed between PC1 scores and sensitisation to any of the major natural and recombinant HDM, cockroach and peanut components on ISAC

(**Figure 6C-E**).

Reactivity to core α-1,3-fucose substituted N-glycans is inversely associated with asthma

No associations were observed between glycan microarray-assessed IgE response PC scores and allergic effector responses (asthma or SPT reactivity (**Figure 6I-J**). Assessment of associations between asthma and responses to individual glycans on the array showed that asthmatics, compared to controls, mounted significantly lower responses to N-glycans carrying core α-1,3-fucose only or in combination with α-1,6-fucose, but not to any other structures on the array (**Figure 7**).

Figure 6. *Associations between anti-glycan IgE responses and atopic sensitisation and asthma*



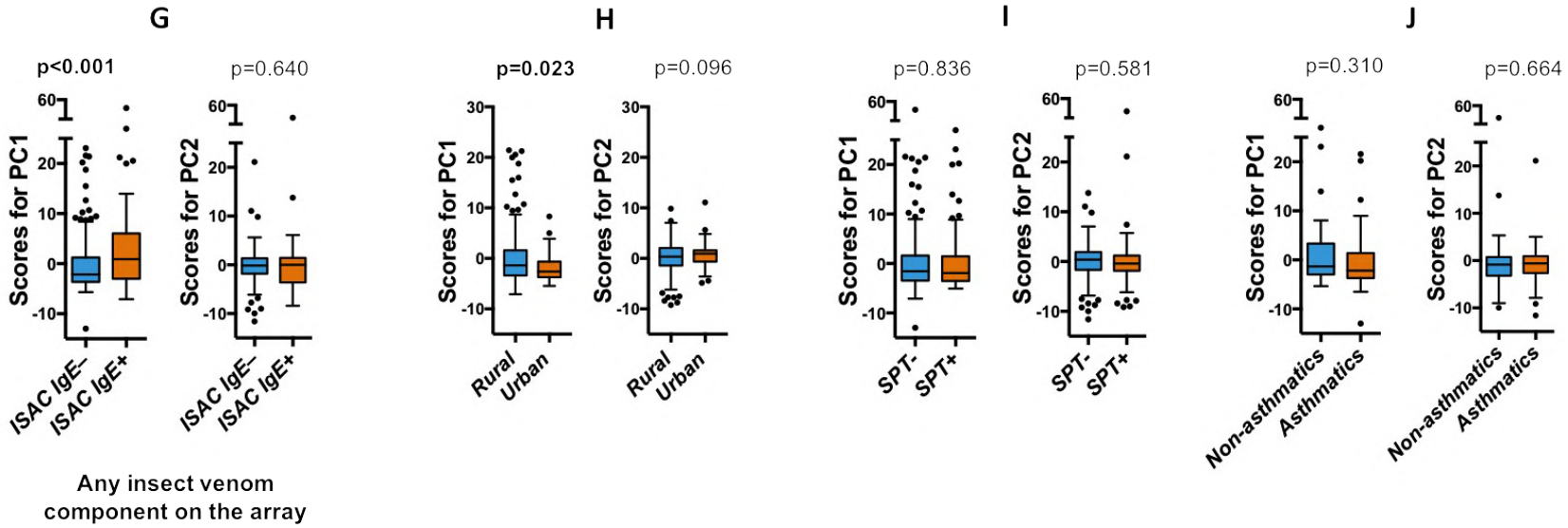
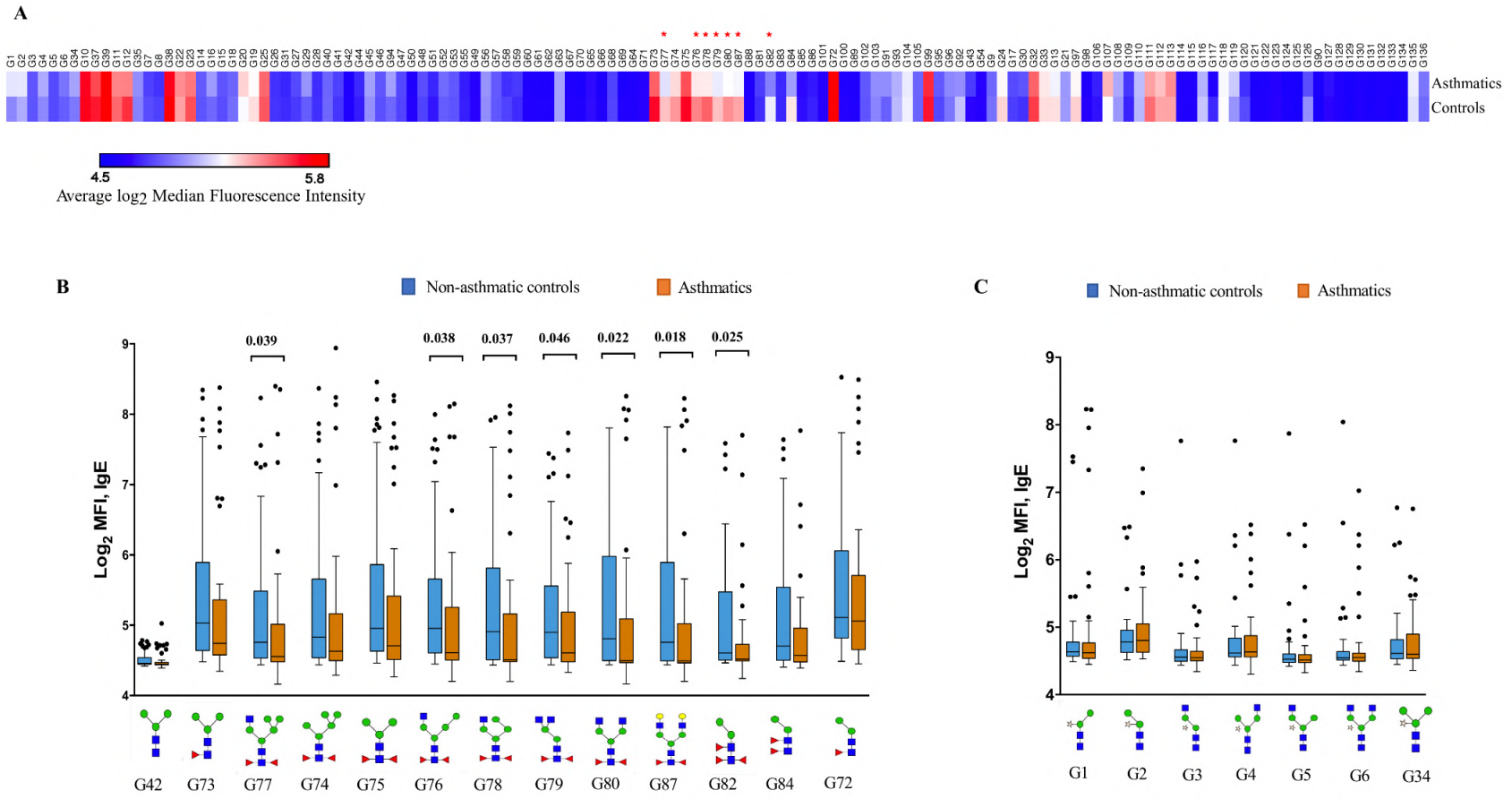


*Figure shows data combined from all three studies. Figure **6A** shows a scatterplot of first (PC1) and second factor (PC2) loadings derived from principal component analysis of IgE responses to 135 synthetic N-glycans. Box-and-whisker plots show associations between PC scores and **(B)** S. mansoni infection [Kato-Katz and/or PCR, infected n=88; uninfected n=154], **(C)** crude D. pteronyssinus extract-specific IgE sensitisation (ImmunoCAP IgE≥0.35 kU/L) and sensitisation (IgE≥0.3 ISU) to any of the dust mite components on the ISAC array, **(D)** crude German cockroach extract-specific IgE sensitisation and sensitisation to any of the German cockroach components on the ISAC array, **(E)** crude peanut (A. hypogaea) extract-specific IgE sensitisation and sensitisation to any of the peanut components on the ISAC array, **(F)** IgE sensitisation (IgE≥0.30 ISU) to any of the CCD-bearing components on the ISAC array, **(G)** IgE sensitisation to any of the insect venom components on the ISAC array, **(H)** the rural-urban environment, **(I)** SPT reactivity to any of Dermatophagoides mix, German cockroach or Blomia tropicalis, **(J)** asthma status.*
Horizontal lines in the plots represent medians and boxes denote interquartile ranges (IQR). Whiskers were drawn using the Tukey method (1.5 times IQR). Individual points represent outliers (>1.5 times IQR away from median).
Associations between PC scores and the various comparison groups were assessed using linear regression analysis in Stata 13.1. Age- and sex-adjusted p values are shown.
***PC1:** Principal Component 1; **PC2:** Principal Component 2; **Sm:** S. mansoni infection determined by Kato-Katz and/or PCR; **ISAC:** Immuno Solid-phase Allergen Chip; **CCD:** cross-reactive carbohydrate determinant; **SPT**: skin prick test*

Figure 7. *Associations between asthma and IgE reactivity to glycans*



(A) *Heatmap of average log_2-transformed MFIs representing IgE reactivity to each individual glycan structure on the array, in asthmatic versus non-asthmatic schoolchildren.* *******$p<0.05$. ***(B)*** *Box-and-whisker plots showing MFIs representing IgE reactivity to core α-1,3-fucosylated N-glycan structures.* ***(C)*** *Box-and-whisker plots showing MFIs representing IgE reactivity to core β-1,2-xylosylated N-glycan structures. Horizontal lines in the plots represent medians and boxes denote interquartile ranges (IQR). Whiskers were drawn using the Tukey method (1.5 times IQR). Individual points represent outliers (> 1.5 times IQR away from median). The Mann-Whitney U test was conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations (in order to adjust for multiple testing), to assess differences between asthmatic and non-asthmatic schoolchildren.*

DISCUSSION

We conducted a component-resolved assessment of associations between IgE responses and allergy outcomes among Ugandan rural and urban individuals, and among asthmatic schoolchildren and their controls. We hypothesised that anti-CCD IgE might contribute to protection against clinical allergy, and found that there was a specific inverse association between reactivity to core α-1,3-fucose substituted N-glycans and asthma. We also found that among rural, urban and non-asthmatic participants, sensitisation to extracts from common environmental allergens did not reflect sensitisation to their established major allergenic components; rather, many participants recognised CCDs and venom proteins, both known to be expressed on *Sm* antigens. By contrast, a high proportion of asthmatics were sensitised to both HDM extract and its major components, lending substantial support to increasing evidence that much asthma in tropical LICs is atopic, contrary to earlier perception.[59,60] Detailed assessment of IgE reactivity to core β-1,2-xylose and/or α-1,3-fucose (well-known, non-mammalian, "classical" CCDs) using a synthetic glycan microarray revealed strong positive associations with *Sm* infection, the rural environment and ImmunoCAP-determined allergen extract-specific IgE sensitisation, but not with ISAC-determined sensitisation to major natural and non-glycosylated recombinant components of these allergen extracts.

Allergen extracts contain a mixture of allergenic and non-allergenic components,[61] as well as cross-reactive protein and carbohydrate components that are conserved in other environmental antigens such as those from schistosomes.[62] This obscures the identity of the molecular drivers, and hence interpretation, of atopic sensitisation, which is regularly diagnosed using allergen extract-based *in vivo* SPTs and/or *in vitro* extract-specific IgE blood immunoassays. Our results show that this has important implications for diagnosis of atopy and use of population attributable fractions to assess the contribution of atopy to allergy-related disease in tropical LICs.

The high prevalence of sensitisation to allergen extracts but low sensitisation to their major allergenic components in our high-*Sm*-transmission rural setting is reminiscent of previous findings by Hamid *et al.* among Indonesian schoolchildren from areas endemic for soil-transmitted helminths (STH) but not schistosomes.[35] Hamid's results imply that cross-reactive protein motifs and/or CCDs from environmental antigens other than schistosomes play an important role in allergy epidemiology. While exposure to STH might theoretically contribute, expression of "classical" CCD epitopes in most STH is not yet well mapped, with STH glycomes understudied compared to the *Schistosoma* glycome. However, absence of significant associations between anti-CCD IgE and infection with any STH in our studies opposes the notion that STH express "classical" CCDs.

Sensitisation to components bearing classical CCDs in our studies was more common than sensitisation to major allergenic components, suggesting that in this setting, IgE to allergen extracts is raised predominantly against carbohydrate groups shared with environmental antigens, such as helminths. There is evidence showing that cross-sensitisation between *Schistosoma* antigens and peanut, pollen or insect venom allergens is predominantly caused by CCDs.[36,63,64] Our results also imply that CCDs contribute considerably to sensitisation to HDM and cockroach allergens in helminth-endemic settings.

Although insect venom N-glycans carry core α-1,3-fucose motifs[65,66] (which, akin to core β-1,2-xylose, are expressed on *Schistosoma* egg N-glycans[17]), this is unlikely to explain why responses to wasp venom components were elevated among rural and *Sm* infected participants, as the ISAC array contains recombinant forms of these components. The observed responses possibly reflect sensitisation to Venom-Allergen-Like (VAL) proteins expressed by schistosomes[67] and other helminths such as hookworm[68] and *Brugia malayi*.[69] Screening the *Sm* GeneDB database (http://www.genedb.org/Homepage/Smansoni) for protein homology between *Sm* and

wasp venoms yields 46 *Sm* protein sequences producing high-scoring segment pairs with up to 43% identity and 56% similarity to Pol d 5, and 41 *Sm* proteins with up to 42% identity and 78% similarity to Ves v 5.

Higher levels of IgE to CCDs in rural compared to urban participants, and among *Sm* infected compared to uninfected participants, imply that *Sm* infection in our setting contributes considerably to high anti-CCD IgE levels and consequently to high prevalence of ImmunoCAP sensitisation to allergen extracts expressing these CCDs. It is likely that previously observed positive associations between *Sm* and HDM extract-specific IgE in our rural setting[5] are partially attributable to CCD-sensitisation. In the same population, prevalence of clinical allergies is low, supporting the hypothesis that helminths may protect against allergic effector responses. The role of CCDs in protection against allergy, if any, is unclear. Alpha-1,3-gal has been implicated in meat allergy;[33] however, our studies did not find any evidence of this, despite elevated α-1,3-gal-specific IgE concentrations. Other than α-1,3-gal-specific IgE, carbohydrate-specific IgE rarely, if ever, translates into clinical allergy.[10,29,34,36] We postulated that elevated IgE responses to specific immunogenic glycans during chronic *Sm* infection might dominate over allergen protein-specific IgE, resulting in reduced allergic effector responses. Many native allergens occur as glycoproteins; therefore, prior high exposure to specific CCDs (perhaps resulting from *Sm* infection) may prime initial and recall IgE responses to preferentially target specific CCD rather than the protein epitopes of allergens. Thus in *Sm*-endemic settings, IgE may be less effectively induced against common protein allergens than against CCDs, as implied by responses to components on the ISAC array in the present study.

In our setting, ISAC-determined anti-CCD IgE was not inversely associated with SPT reactivity or asthma, and PCA of microarray-determined anti-glycan IgE responses suggested no overall association (inverse or otherwise) between asthma and anti-glycan IgE. However, analysis of levels of IgE to individual glycans revealed that reactivity to

core α-1,3-fucose substituted N-glycans was lower among asthmatic schoolchildren compared to controls. We now hypothesise that responses to specific glycans are not merely a smokescreen that obscures readouts for assessment of atopy; they may be important in protection against allergic disease through specific, yet-to-be-defined, mechanisms. Further mechanistic investigations in animal and human studies are required to investigate this hypothesis. Current efforts to reconstruct helminth glycoproteins (including those carrying N-glycan core substitutions) in plant systems[70] offer great promise for availability of tailored glycan epitopes for initial testing in animal models.

Overall, our data indicate that in tropical LICs, IgE to allergen extracts (detected in standard ImmunoCAP assays) reflects sensitisation to a myriad of environmental exposures (absent in high-income countries), such as CCDs expressed by some helminths, and may not accurately define allergy-related disease phenotypes. In fact, our findings suggest that reactivity to specific (but not all) CCD epitopes might contribute to protection against clinical allergy.

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Author contributions

AME, MY, GN, HM, RvR, CHH and AvD contributed to the conception and experimental design of the study. AME, RES, HM and MN led the field and clinic procedures. SS and NCR constructed the glycan microarrays. GN conducted the laboratory experiments, with SV, JN, IN and JK making key contributions. GN, ELW and AvD analysed the data. GN wrote the manuscript, with all authors contributing to the interpretation of the results, and revision and approval of the final manuscript.

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7.3 Supplementary information for Research paper 4

7.3.1 Supplementary methods

7.3.1.1 Sample size considerations

In the rural survey, 2961 participants from the 26 study villages had a plasma sample stored and hence provided the sampling frame for selection for the ImmunoCAP IgE test. 780 participants (approximately 30 per village) were randomly selected using Stata 13.1 software (StataCorp, College Station, Texas, U.S.A). The rural survey was the three-year outcome survey of the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA; ISRCTN47196031[1]). The main LaVIISWA trial analysis aimed to compare outcomes between the two trial arms,[1,2] hence a sample size of 780 was expected to give 80% power to detect a 35% relative difference in the prevalence of ImmunoCAP allergen-specific IgE sensitisation between the two trial arms, based on an assumed overall allergen-specific IgE sensitisation prevalence of 50% (from results at baseline), and an estimated coefficient of variation (in IgE levels between clusters) of 0.2.

Samples for the glycan and ISAC microarray experiments were randomly selected from among those with ImmunoCAP data. We initially aimed for 50 samples per trial arm (100 in total), and managed to test a total of 209 (glycan array) and 126 samples (ISAC array experiments, respectively), owing to availability of further funding. Similar numbers have been shown to be useful in published studies that have assessed differences in microarray-assessed antibody levels between helminth infected and uninfected, and allergic and non-allergic individuals,[3,4] and between children and adults.[5]

In the urban survey, 1356 participants had a plasma sample stored. ImmunoCAP data on cockroach- and dust mite-specific IgE were available for rural survey participants, hence calculations could be conducted to estimate how many urban survey plasma samples were required to attain significant differences in the prevalence of ImmunoCAP positivity between the urban and rural survey. Assuming a 35% prevalence of

ImmunoCAP positivity to either cockroach or dust mite in the rural survey and a design effect of 1.3, 353 urban survey participants were required for 80% power to detect an absolute difference in proportion positive of 0.10 between the rural and urban survey. For the glycan and ISAC microarray experiments, a sample size of 50 was deemed sufficient to detect differences between the rural and urban setting, based on previous studies doing similar comparisons.[3]

In the asthma case-control study, 557 cases and 1128 controls had a plasma sample stored. Four hundred participants (200 asthmatics and 200 controls) were randomly selected for the ImmunoCAP test. This number was expected to yield 90% power to detect a significant positive association (at an odds ratio of 2) between asthma and ImmunoCAP positivity to any of dust mite, cockroach or peanut (IgE ≥0.35 kU/L). For glycan and ISAC microarray experiments, a sample size of 50 cases and 50 controls was deemed sufficient, based on numbers used in the rural and the urban survey.

7.3.1.2 ImmunoCAP ISAC® sIgE 112 test

This test was conducted according to the manufacturer's instructions. The ISAC (Immuno Solid-phase Allergen Chip) comprises four reaction sites, each printed with 112 allergen components in a microarray format. Before the immunoassay, each microarray chip was washed to remove any allergens that were non-covalently bound to the microarray surface. Each reaction site was then incubated with 30 µl of undiluted participant plasma for two hours at room temperature in a humidified chamber to enable reactions between IgE and allergen components. Following a washing step, each microarray was incubated for 30 minutes with 30 µl of fluorescence-labeled anti-human IgE detection antibody. Unbound detection antibody was washed off and the fluorescence intensity of each microarray measured using a scanner (LuxScan 10K/A, CapitalBio, Beijing, China). Analysis of the resultant digitalized images was done using Phadia Microarray Image Analysis software (Thermo Fisher Scientific). Results were reported in arbitrary semi-quantitative ISAC Standardised Units (ISU).

7.3.1.3 Microarray detection of N-glycan-specific IgE

Each reaction site on each microarray slide included fluorescently-labeled bovine serum albumin (BSA) as a printing control. The NEXTERION®-coated microarray slides [Schott AG, Germany] used in the IgE binding assay were blocked (during microarray construction) with 50mM ethanolamine in 50mM sodium borate buffer pH 9.0, and stored at -20°C. On the day of the binding assay, they were thawed at room temperature (RT) and covered with silicone gaskets to create seven wells with printed microarrays per slide. Each microarray was incubated with 300 µl of a 1:30 plasma dilution in 1% BSA - 0.01% Tween20 for one hour at RT while shaking. After sequential washes with PBS-0.05% Tween20 and PBS, the slides were incubated for 30 minutes at RT in the dark with PromoFluor 647 (VWR, USA)-labeled anti-human IgE clone MH25-1 (Sanquin, Amsterdam, Netherlands) [diluted 1/150 in PBS-0.01% Tween20], while shaking. After a final wash with PBS-0.05% Tween20, PBS and deionised water, sequentially, the slides were dried and kept in the dark until scanning. The slides were scanned for fluorescence at a 10µm resolution with a G2565BA scanner (Agilent Technologies, CA, USA) using a 633nm laser.

7.3.1.4 Glycan microarray image processing

Using GenePix Pro 7.0 software (Molecular Devices, CA, USA), a spot-finding algorithm was used to align and re-size fluorescence spots in the microarray images, without setting a composite pixel intensity threshold. Data on median fluorescence intensity (MFI) for each spot and the local background were then exported to Microsoft Excel software. In all analyses, MFIs that were highlighted as artefacts by the GenePix Pro 7.0 software were excluded. Further processing of IgE MFIs in Excel was done as described by Amoah *et al.*,[3] as follows: for each IgE spot, the ratio of the MFI of the spot to the local background MFI was obtained and then multiplied by the average of background MFI for all the spots on the array. For each of the structures, the average over four spots (or less, in case of unreliable data that were excluded) was then $\log_2$-transformed.

7.3.2 Supplementary tables and figures

Table S1. List of allergen components on the ImmunoCAP ISAC® microarray (source: Thermo Fisher Scientific, Uppsala, Sweden)

Allergen component	Allergen source	Latin name	Protein group
nAct d 1	Kiwi	*Actinidia deliciosa*	
nAct d 2	Kiwi	*Actinidia deliciosa*	Thaumatine-like protein
nAct d 5	Kiwi	*Actinidia deliciosa*	
rAct d 8	Kiwi	*Actinidia deliciosa*	PR-10 protein
rAln g 1	Alder	*Alnus glutinosa*	PR-10 protein
rAlt a 1	Alternaria	*Alternaria alternata*	
rAlt a 6	Alternaria	*Alternaria alternata*	Enolase
nAmb a 1	Ragweed	*Ambrosia artemisiifolia*	
rAna o 2	Cashew nut	*Anacardium occidentale*	Storage protein, 11S globulin
rAni s 1	Anisakis	*Anisakis simplex*	
rAni s 3	Anisakis	*Anisakis simplex*	Tropomyosin
rApi g 1	Celery	*Apium graveolens*	PR-10 protein
rApi m 1	Honey bee venom	*Apis mellifera*	Phospholipase A2
nApi m 4	Honey bee venom	*Apis mellifera*	Melittin
rAra h 1	Peanut	*Arachis hypogaea*	Storage protein ,7S globulin
rAra h 2	Peanut	*Arachis hypogaea*	Storage protein, Conglutin
rAra h 3	Peanut	*Arachis hypogaea*	Storage protein, 11S globulin
nAra h 6	Peanut	*Arachis hypogaea*	Storage protein, Conglutin
rAra h 8	Peanut	*Arachis hypogaea*	PR-10 protein
rAra h 9	Peanut	*Arachis hypogaea*	Lipid transfer protein (nsLTP)
nArt v 1	Mugwort	*Artemisia vulgaris*	
nArt v 3	Mugwort	*Artemisia vulgaris*	Lipid transfer protein (nsLTP)
rAsp f 1	Aspergillus	*Aspergillus fumigatus*	
rAsp f 3	Aspergillus	*Aspergillus fumigatus*	
rAsp f 6	Aspergillus	*Aspergillus fumigatus*	Mn superoxide dismutase
rBer e 1	Brazil nut	*Bertholletia excelsa*	Storage protein, 2S albumin
rBet v 1	Birch	*Betula verrucosa*	PR-10 protein
rBet v 2	Birch	*Betula verrucosa*	Profilin
rBet v 4	Birch	*Betula verrucosa*	Polcalcin
rBla g 1	Cockroach	*Blattella germanica*	
rBla g 2	Cockroach	*Blattella germanica*	
rBla g 5	Cockroach	*Blattella germanica*	

nBla g 7	Cockroach	*Blattella germanica*	Tropomyosin
rBlo t 5	House dust mite	*Blomia tropicalis*	
nBos d 4	Cow's milk	*Bos domesticus*	Alpha-lactalbumin
nBos d 5	Cow's milk	*Bos domesticus*	Beta-lactoglobulin
nBos d 6	Cow's milk and meat	*Bos domesticus*	Serum albumin
nBos d 8	Cow's milk	*Bos domesticus*	Casein
nBos d lactoferrin	Cow's milk	*Bos domesticus*	Transferrin
rCan f 1	Dog	*Canis familiaris*	Lipocalin
rCan f 2	Dog	*Canis familiaris*	Lipocalin
nCan f 3	Dog	*Canis familiaris*	Serum albumin
rCan f 5	Dog	*Canis familiaris*	Arginine esterase
rChe a 1	Goosefoot	*Chenopodium album*	
rCla h 8	Cladosporium	*Cladosporium herbarum*	
rCor a 1.0101	Hazel pollen	*Corylus avellana*	PR-10 protein
rCor a 1.0401	Hazelnut	*Corylus avellana*	PR-10 protein
rCor a 8	Hazelnut	*Corylus avellana*	Lipid transfer protein (nsLTP)
nCor a 9	Hazelnut	*Corylus avellana*	Storage protein, 11S globulin
nCry j 1	Japanese ceder	*Cryptomeria japonica*	
nCup a 1	Cypress	*Cupressus arizonica*	
nCyn d 1	Bermuda grass	*Cynodon dactylon*	Grass group 1
nDer f 1	House dust mite	*Dermatophagoides farinae*	
rDer f 2	House dust mite	*Dermatophagoides farinae*	
nDer p 1	House dust mite	*Dermatophagoides pteronyssinus*	
rDer p 10	House dust mite	*Dermatophagoides pteronyssinus*	Tropomyosin
rDer p 2	House dust mite	*Dermatophagoides pteronyssinus*	
rEqu c 1	Horse	*Equus caballus*	Lipocalin
nEqu c 3	Horse	*Equus caballus*	Serum albumin
nFag e 2	Buckwheat	*Fagopyrum esculentum*	Storage protein, 2S albumin
rFel d 1	Cat	*Felis domesticus*	Uteroglobin
nFel d 2	Cat	*Felis domesticus*	Serum albumin
rFel d 4	Cat	*Felis domesticus*	Lipocalin
rGad c 1	Cod	*Gadus callarias*	Parvalbumin
nGal d 1	Egg white	*Gallus domesticus*	Ovomucoid

nGal d 2	Egg white	*Gallus domesticus*	Ovalbumin
nGal d 3	Egg white	*Gallus domesticus*	Conalbumin/Ovotransferrin
nGal d 5	Egg yolk/chicken meat	*Gallus domesticus*	Livetin/Serum albumin
rGly m 4	Soybean	*Glycine max*	PR-10 protein
nGly m 5	Soybean	*Glycine max*	Storage protein, Beta-conglycinin
nGly m 6	Soybean	*Glycine max*	Storage protein, Glycinin
rHev b 1	Latex	*Hevea brasiliensis*	
rHev b 3	Latex	*Hevea brasiliensis*	
rHev b 5	Latex	*Hevea brasiliensis*	
rHev b 6.01	Latex	*Hevea brasiliensis*	
rHev b 8	Latex	*Hevea brasiliensis*	Tropomyosin
nJug r 1	Walnut	*Juglans regia*	Storage protein, 2S albumin
nJug r 2	Walnut	*Juglans regia*	Storage protein, 7S globulin
nJug r 3	Walnut	*Juglans regia*	Lipid transfer protein (nsLTP)
rLep d 2	Storage mite	*Lepidoglyphus destructor*	
rMal d 1	Apple	*Malus domestica*	PR-10 protein
rMer a 1	Annual mercury	*Mercurialis annua*	Profilin
nMus m 1	Mouse	*Mus musculus*	Lipocalin
nMUXF3	Sugar epitope from Bromelain		Tropomyosin
nOle e 1	Olive	*Olea europaea*	
nOle e 7	Olive	*Olea europaea*	Lipid transfer protein (nsLTP)
rOle e 9	Olive	*Olea europaea*	
rPar j 2	Wall pellitory	*Parietaria judaica*	Lipid transfer protein (nsLTP)
nPen m 1	Shrimp	*Penaeus monodon*	Tropomyosin
nPen m 2	Shrimp	*Penaeus monodon*	Arginine kinase
nPen m 4	Shrimp	*Penaeus monodon*	Sarcoplasmic Ca-binding protein
rPhl p 1	Timothy grass	*Phleum pratense*	Grass group 1
rPhl p 11	Timothy grass	*Phleum pratense*	
rPhl p 12	Timothy grass	*Phleum pratense*	Profilin
rPhl p 2	Timothy grass	*Phleum pratense*	Grass group 2
nPhl p 4	Timothy grass	*Phleum pratense*	
rPhl p 5	Timothy grass	*Phleum pratense*	Grass group 5
rPhl p 6	Timothy grass	*Phleum pratense*	
rPhl p 7	Timothy grass	*Phleum pratense*	Polcalcin
rPla a 1	Plane tree	*Platanus acerifolia*	
nPla a 2	Plane tree	*Platanus acerifolia*	

rPla a 3	Plane tree	*Platanus acerifolia*	Lipid transfer protein (nsLTP)
rPla l 1	Plantain (English)	*Plantago lanceolata*	
rPol d 5	Paper wasp venom	*Polistes dominulus*	Venom, Antigen 5
rPru p 1	Peach	*Prunus persica*	PR-10 protein
rPru p 3	Peach	*Prunus persica*	Lipid transfer protein (nsLTP)
nSal k 1	Saltwort	*Salsola kali*	
nSes i 1	Sesame seed	*Sesamum indicum*	Storage protein, 2S albumin
rTri a 14	Wheat	*Triticum aestivum*	Lipid transfer protein (nsLTP)
rTri a 19.0101	Wheat	*Triticum aestivum*	Omega-5 gliadin
nTri a aA_TI	Wheat	*Triticum aestivum*	
rVes v 5	Common wasp venom	*Vespula vulgaris*	Venom, Antigen 5

Figure S1. Collection of synthetic structural variants on the glycan microarray

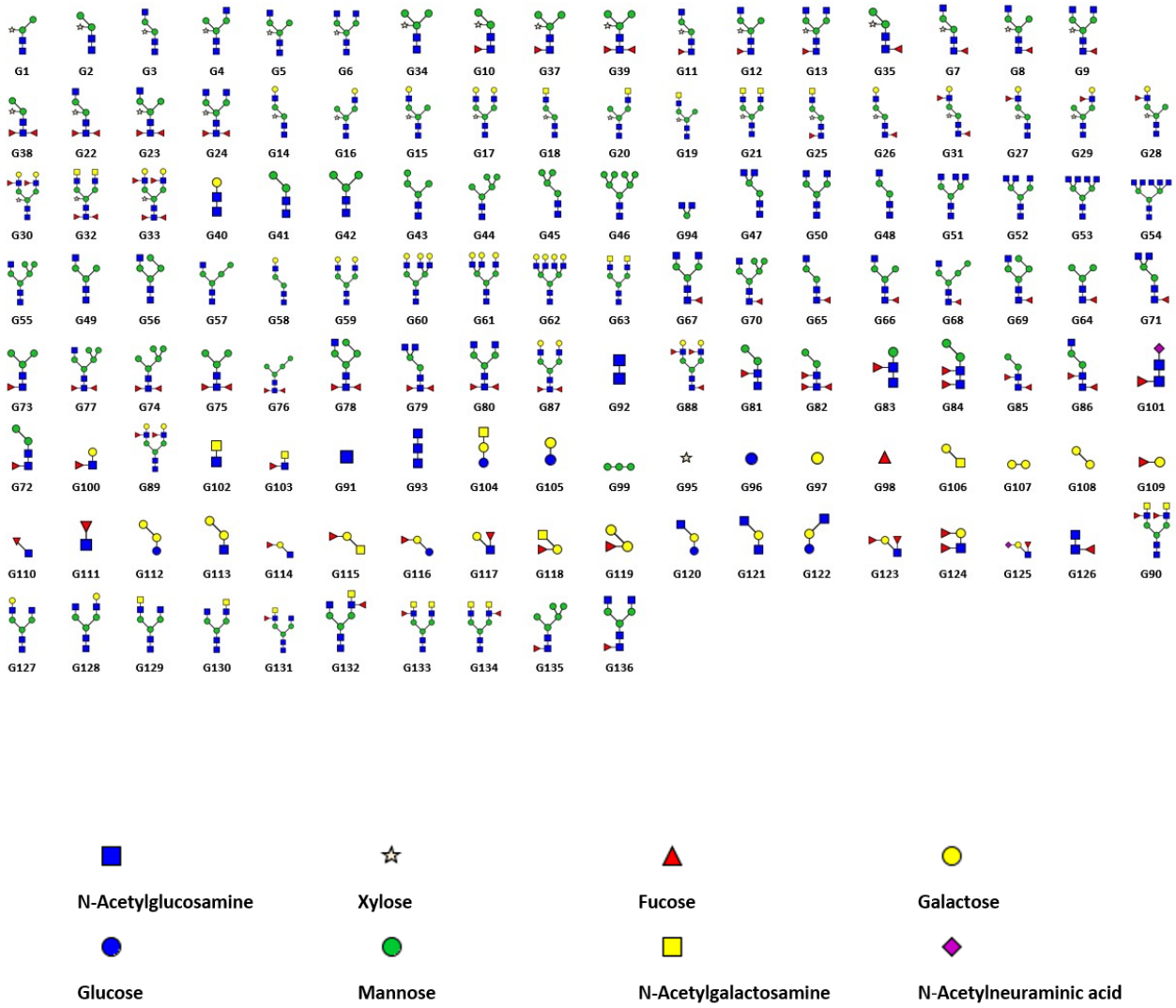

Table S2. Impact of community-based intensive versus standard anthelminthic treatment on IgE profiles in the rural survey

Outcome	n/N (%) / geometric mean		Unadjusted		Adjusted for age and sex	
	Standard	**Intensive**	**RR[§] / GMR[#] (95% CI)**	**p-value**	**RR[§] / GMR[#] (95% CI)**	**p-value**
ImmunoCAP-determined IgE						
Dermatophagoides, cockroach or peanut positivity (IgE≥0.35kU/L)	223/390 (57.2%)	214/390 (54.9%)	0.95 (0.81, 1.10)	0.46	0.94 (0.80, 1.10)	0.41
Dermatophagoides positivity (IgE>0.35kU/L)	134/390 (34.4%)	130/390 (33.3%)	0.95 (0.76, 1.20)	0.67	0.96 (0.77, 1.19)	0.68
German cockroach positivity (IgE>0.35kU/L)	201/390 (51.5%)	192/390 (49.2%)	0.94 (0.80, 1.11)	0.47	0.94 (0.79, 1.11)	0.42
Peanut positivity (IgE>0.35kU/L)	59/390 (15.1%)	55/390 (14.1%)	0.92 (0.59, 1.41)	0.68	0.91 (0.58, 1.41)	0.66
Concentration of IgE to *Dermatophagoides*	GM: 0.158	GM: 0.129	0.78 (0.51, 1.17)	0.22	0.76 (0.51, 1.13)	0.17
Concentration of IgE to cockroach	GM: 0.342	GM: 0.289	0.82 (0.55, 1.22)	0.31	0.81 (0.55, 1.20)	0.28
Concentration of IgE to peanut	GM: 0.074	GM: 0.066	0.89 (0.64, 1.23)	0.47	0.89 (0.65, 1.23)	0.49
ISAC-determined IgE sensitisation (IgE≥0.3 ISU)						
CCD-bearing components	9/72 (12.5%)	12/54 (22.2%)	1.87 (0.79, 4.41)	0.13	2.46 (0.92, 6.57)	0.12
Venoms	11/72 (15.3%)	16/54 (29.6%)	1.87 (0.90, 3.90)	0.10	1.23 (0.55, 2.79)	0.59
Dust mites	1/72 (1.4%)	3/54 (5.7%)	2.80 (0.23, 33.85)	0.41	3.3 (0.27, 40.80)	0.36
Glycan-specific IgE mean fluorescence intensity						

 G42	GM: 4.778	GM: 4.760	0.99 (0.99, 1.00)	0.34	0.99 (0.99, 1.00)	0.24
 G34	GM: 4.847	GM: 4.834	1.02 (0.98, 1.06)	0.44	1.02 (0.98, 1.06)	0.31
 G73	GM: 5.070	GM: 5.106	1.01 (0.96, 1.06)	0.79	1.01 (0.96, 1.07)	0.61
 G37	GM: 5.205	GM: 5.322	1.02 (0.96, 1.09)	0.50	1.03 (0.97, 1.10)	0.34

[§]Risk ratios (mean of the cluster proportions in intensive arm divided by the mean of the cluster proportions in standard arm) were calculated to assess the impact of trial intervention on ImmunoCAP-determined IgE sensitisation to crude house dust mite, German cockroach and peanut, and on ISAC-determined sensitisation to at least one allergen component within the larger allergen groupings of CCDs, venoms, and dust mites.

[#]Geometric mean ratios were calculated to assess the impact of trial intervention on the concentration of ImmunoCAP-determined IgE sensitisation to individual allergens and on IgE mean fluorescence intensities to representative glycans on the array.

***RR**: risk ratio; **GM:** geometric mean; **GMR:** geometric mean ratio; **CI:** confidence interval; **ISAC**: Immuno Solid-phase Allergen Chip; **ISU**: ISAC standardised units*

Table S3. Differences in ISAC-determined IgE reactivity in the rural versus urban setting and among asthmatics versus non-asthmatics

Component	% with detectable IgE, ISAC			% with IgE≥0.30 ISU, ISAC			% with detectable IgE, ISAC			% with IgE≥0.30 ISU, ISAC		
	Rural (n=126)	Urban (n=60)	p	Rural (n=126)	Urban (n=60)	p	Non-asthmatics (n=50)	Asthmatics (n=50)	p	Non-asthmatics (n=50)	Asthmatics (n=50)	p
CCD-bearing components												
nJug r 2	23.8	25.0	0.859	9.5	5.0	0.225	36.0	38.0	0.836	10.0	16.0	0.375
nMUXF3	11.1	5.0	0.139	4.8	1.7	0.279	18.0	18.0	1.000	10.0	6.0	0.463
nPhl p 4	**28.6**	10	**0.005**	14.3	8.3	0.181	34.0	32.0	0.832	16.0	12.0	0.566
nCry j 1	**9.5**	1.7	**0.040**	2.4	1.7	0.612	8.0	14.0	0.340	2.0	10.0	0.094
nCup a 1	8.7	3.3	0.148	2.4	1.7	0.612	12.0	14.0	0.767	4.0	12.0	0.142
nCyn d 1	19.8	10.0	0.067	11.1	6.7	0.249	24.0	24.0	1.000	16.0	18.0	0.791
nPla a 2	13.5	6.7	0.128	**6.3**	0.0	**0.041**	26.0	24.0	0.818	8.0	10.0	0.728
Venoms												
rPol d 5	**39.7**	20	**0.008**	19.0	11.7	0.207	38.0	40.0	0.838	26.0	22.0	0.641
rVes v 5	**24.6**	10	**0.001**	9.5	3.0	0.225	32.0	26.0	0.511	20.0	8.0	0.085
rApi m 1	5.6	5.0	0.207	4.8	1.0	0.279	**10.0**	**0.0**	**0.023**	0.0	0.0	
nApi m 4	4.8	0.0	0.090	1.6	0.0	0.458	2.0	0.0	0.317	0.0	0.0	
Cross-reactive protein components												
Tropomyosins												
nBla g 7	4.0	1.7	0.369	2.4	1.7	0.612	8.0	12.0	0.507	2.0	10.0	0.094
rDer p 10	3.9	1.7	0.369	3.2	1.7	0.481	6.0	12.0	0.296	2.0	10.0	0.094
nPen m1	4.8	5.0	0.600	4.0	1.7	0.369	12.0	12.0	1.000	4.0	12.0	0.142
rAni s 3	5.6	3.3	0.400	3.2	1.7	0.549	4.0	10.0	0.242	2.0	8.0	0.171
rHev b 8	0.8	1.7	0.542	0.8	1.7	0.542	4.0	0.0	0.155	2.0	0.0	0.317
Polcalcins												
rBet v 4	0.0	0.0		0.0	0.0		0.0	2.0	0.500	0.0	0.0	
rPhl p 7	0.8	0.0	0.677	0.0	0.0		2.0	2.0	0.753	0.0	2.0	0.500
Profilins												
rPhl p 12	1.6	0.0	0.458	0.0	0.0		2.0	0.0	0.500	0.0	0.0	
rBet v 2	0.0	1.7	0.323	0.0	0.0		2.0	0.0	0.500	2.0	0.0	0.500
rMer a 1	0.8	1.7	0.542	0.0	0.0		2.0	2.0	0.753	2.0	0.0	0.500

Dust mites												
nDer p 1	2.4	8.3	0.073	2.4	5.0	0.296	16.0	28.0	0.149	12.0	26.0	0.075
rDer p 2	2.4	**13.3**	**0.006**	1.6	**10.0**	**0.015**	10.0	**36.0**	**0.002**	8.0	**36.0**	**<0.001**
nDer f 1	6.3	5.0	0.502	2.4	1.7	0.612	16.0	28.0	0.149	12.0	22.0	0.185
rDer f 2	2.4	8.3	0.073	1.6	**8.3**	**0.036**	14.0	**36.0**	**0.012**	8.0	**36.0**	**<0.001**
rBlo t 5	0.8	**6.7**	**0.038**	0.0	**5.0**	**0.032**	8.0	20.0	0.085	4.0	**18.0**	**0.026**
rLep d 2	0.0	**6.6**	**0.010**	0.0	**6.7**	**0.010**	12.0	**32.0**	**0.016**	8.0	**24.0**	**0.029**
Cockroach												
rBla g 1	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
rBla g 2	0.0	1.7	0.323	0.0	0.0		2.0	10.0	0.102	0.0	2.0	0.500
rBla g 5	0.8	0.0	0.677	0.0	0.0		0.0	2.0	0.500	0.0	0.0	
Food allergens												
rAna o 2	4.8	6.7	0.411	2.4	3.3	0.519	4.0	4.0	0.691	2.0	0.0	0.500
rApi g 1	2.4	**10.0**	**0.033**	0.0	0.0		0.0	0.0		0.0	0.0	
nPen m2	4.8	5.0	0.600	5.6	6.7	0.498	16.0	16.0	1.000	10.0	12.0	0.500
nPen m 4	0.8	0.0	0.677	0.0	0.0		6.0	2.0	0.309	4.0	2.0	0.500
nBos d 4	0.8	0.0	0.677	0.0	0.0		2.0	4.0	0.500	2.0	0.0	0.500
nBos d 6	0.0	0.0		0.0	0.0		10.0	6.0	0.357	0.0	0.0	
nBos d 8	3.2	0.0	0.207	1.6	0.0	0.458	0.0	0.0		0.0	0.0	
nBos d lactoferrin	2.4	0.0	0.308	0.0	0.0		8.0	12.0	0.370	4.0	4.0	0.691
nGal d 1	3.9	8.3	0.186	0.0	3.3	0.103	2.0	0.0	0.500	2.0	0.0	0.500
nGal d 2	3.2	3.3	0.631	0.8	0.0	0.677	0.0	0.0		0.0	0.0	
nGal d 3	0.8	3.3	0.244	0.8	1.7	0.542	2.0	0.0	0.500	0.0	0.0	
nGal d 5	3.9	0.0	0.139	0.8	0.0	0.677	0.0	2.0	0.500	0.0	0.0	
rGly m 4	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
nGly m 5	0.0	0.0		0.0	0.0		2.0	0.0	0.500	2.0	0.0	0.500
nGly m 6	0.0	1.7	0.323	0.0	0.0		6.0	6.0	0.661	2.0	0.0	0.500
rMal d 1	0.8	**13.3**	**0.001**	0.0	0.0		10.0	4.0	0.218	6.0	0.0	0.121
rPru p 1	3.9	3.3	0.596	1.6	1.7	0.692	6.0	4.0	0.500	0.0	0.0	
rPru p 3	0.0	0.0		0.0	0.0		0.0	2.0	0.500	0.0	0.0	
rTri a 14	2.4	0.0	0.308	0.0	0.0		0.0	0.0		0.0	0.0	
rTri a 19.0101	7.1	5.0	0.420	1.6	1.7	0.692	0.0	0.0		0.0	0.0	
nTri a aA_TI	0.8	0.0	0.677	0.0	0.0		2.0	4.0	0.500	0.0	0.0	
nAct d 1	0.8	0.0	0.677	0.8	0.0	0.677	6.0	0.0	0.121	2.0	0.0	0.500
nAct d 2	0.8	0.0	0.677	0.0	0.0		0.0	0.0		0.0	0.0	

nAct d 5	0.8	0.0	0.677	0.8	0.0	0.677	0.0	0.0		0.0	0.0	
rAct d 8	1.6	1.6	0.692	0.8	0.0	0.677	8.0	4.0	0.339	4.0	0.0	0.247
rAra h 1	0.8	0.0	0.677	0.0	0.0		0.0	4.0	0.247	0.0	0.0	
rAra h 2	0.8	0.0	0.677	0.0	0.0		0.0	0.0		0.0	0.0	
rAra h 3	1.6	0.0	0.458	1.6	0.0	0.458	0.0	0.0		0.0	0.0	
nAra h 6	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
rAra h 8	0.0	3.3	0.103	0.0	0.0		0.0	6.0	0.121	0.0	0.0	
rAra h 9	0.8	0.0	0.677	0.0	0.0		0.0	2.0	0.500	0.0	0.0	
rBer e 1	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
rCor a 1.0401	0.0	1.7	0.323	0.0	0.0		2.0	0.0	0.500	0.0	0.0	
rCor a 8	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
nCor a 9	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
nJug r 1	0.0	0.0		0.0	0.0		0.0	2.0	0.500	0.0	0.0	
nJug r 3	0.0	0.0		0.0	0.0		2.0	0.0	0.500	2.0	0.0	0.500
rPla l 1	0.0	0.0		0.0	0.0		6.0	0.0	0.121	2.0	0.0	0.500
nSes i 1	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
nFag e 2	0.0	0.0		0.0	0.0		2.0	0.0	0.500	2.0	0.0	0.500
Pollen												
rAln g 1	0.8	0.0	0.677	0.0	0.0		0.0	0.0		0.0	0.0	
nArt v 1	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
nArt v 3	0.0	0.0		0.0	0.0		2.0	0.0	0.500	0.0	0.0	
rBet v 1	1.6	0.0	0.458	0.0	0.0		10.0	8.0	0.500	4.0	0.0	0.247
rChe a 1	0.8	0.0	0.677	0.0	0.0		2.0	4.0	0.500	0.0	0.0	
rMer a 1	0.8	1.7	0.542	0.0	0.0		2.0	2.0	0.753	2.0	0.0	0.500
nOle e 7	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
rOle e 9	2.4	3.3	0.519	0.0	0.0		0.0	4.0	0.247	0.0	2.0	0.500
rPar j 2	0.0	0.0		0.0	0.0		2.0	4.0	0.500	0.0	2.0	0.500
rCor a 1.0101	2.4	3.3	0.519	0.8	1.7	0.542	4.0	6.0	0.500	0.0	2.0	0.500
rPhl p 1	0.0	1.7	0.323	0.0	0.0		6.0	2.0	0.309	4.0	0.0	0.247
rPhl p 11	0.8	1.7	0.542	0.8	0.0	0.677	0.0	4.0	0.247	0.0	0.0	
rPhl p 12	1.6	0.0	0.458	0.0	0.0		2.0	0.0	0.500	0.0	0.0	
rPhl p 2	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
rPhl p 5	0.8	0.0	0.677	0.0	0.0		4.0	0.0	0.247	0.0	0.0	
rPhl p 6	1.6	1.7	0.692	0.0	1.7	0.323	0.0	0.0		0.0	0.0	
rPhl p 7	0.8	0.0	0.677	0.0	0.0		2.0	2.0	0.753	0.0	2.0	0.500
rPla a 1	6.4	3.3	0.319	3.2	1.7	0.481	10.0	6.0	0.357	4.0	2.0	0.500

rPla a 3		0.0	0.0		0.0	0.0			0.0	0.0		0.0	0.0	
nSal k 1		0.8	0.0	0.677	0.8	0.0	0.677		4.0	10.0	0.218	0.0	4.0	0.247
Fungi														
rAlt a 1		0.0	0.0		0.0	0.0			0.0	0.0		0.0	0.0	
rAlt a 6		1.6	3.3	0.388	0.8	1.7	0.542		4.0	4.0	0.691	0.0	0.0	
nAmb a 1		0.8	0.0	0.677	0.0	0.0			0.0	0.0		0.0	0.0	
rAsp f 1		5.6	8.3	0.334	0.8	0.0	0.677		4.0	0.0	0.247	0.0	0.0	
rAsp f 3		6.4	11.7	0.169	1.6	1.7	0.692		2.0	2.0	0.753	0.0	0.0	
rAsp f 6		3.9	1.7	0.369	1.6	0.0	0.458		4.0	0.0	0.247	4.0	0.0	0.247
rCla h 8		0.8	0.0	0.677	0.0	0.0			0.0	2.0	0.500	0.0	0.0	
Other, animal														
rAni s 1		0.0	0.0		0.0	0.0			2.0	0.0	0.500	2.0	0.0	0.500
rCan f 2		0.8	1.7	0.542	0.0	0.0			0.0	0.0		0.0	0.0	
nCan f 3		0.0	0.0		0.0	0.0			0.0	4.0	0.247	0.0	0.0	
rCan f 5		2.4	1.7	0.612	0.8	0.0	0.677		4.0	0.0	0.247	2.0	0.0	0.500
rEqu c 1		0.8	0.0	0.677	0.0	0.0			0.0	0.0		0.0	0.0	
nEqu c 3		0.0	0.0		0.0	0.0			0.0	2.0	0.500	0.0	0.0	
rFel d 1		0.0	0.0		0.0	0.0			0.0	0.0		0.0	0.0	
nFel d 2		2.4	6.7	0.153	0.0	0.0			6.0	10.0	0.357	2.0	0.0	0.500
rFel d 4		**0.8**	**6.7**	**0.038**	0.0	0.0			2.0	2.0	0.753	0.0	2.0	0.500
nMus m 1		0.0	0.0		0.0	0.0			2.0	6.0	0.309	2.0	4.0	0.500
Latex														
rHev b 1		1.6	1.7	0.692	1.6	1.7	0.692		2.0	0.0	0.500	0.0	0.0	
rHev b 3		0.8	1.7	0.542	0.8	1.7	0.542		0.0	0.0		0.0	0.0	
rHev b 5		0.8	0.0	0.677	0.0	0.0			0.0	0.0		0.0	0.0	
rHev b 6.01		1.6	1.7	0.692	0.8	1.7	0.542		2.0	0.0	0.500	2.0	0.0	0.500
rHev b 8		0.8	1.7	0.542	0.8	1.7	0.542		4.0	0.0	0.247	2.0	0.0	0.500

Table shows proportions of participants that had an ISAC-detectable IgE response or were sensitised (IgE≥0.3 ISU) to components on the array. The Chi-square / Fisher's exact test was conducted to assess differences in proportions between rural and urban individuals, and between asthmatics and their controls. These tests were conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations in order to adjust for multiple testing.
***ISAC**: Immuno Solid-phase Allergen Chip; **ISU**: ISAC standardised units*

Table S4. ISAC-determined IgE reactivity among helminth infected and uninfected rural survey participants

Allergen group	% with detectable IgE, ISAC array			% with IgE≥0.30 ISU, ISAC array		
	Any worm infection (n=62)	**Uninfected (n=50)**	p	**Any worm infection (n=62)**	**Uninfected (n=50)**	p
CCD-bearing components	38.7	26.0	0.155	17.7	14.0	0.592
Venoms	59.7	32.0	**0.004**	30.7	12.0	**0.018**
Dust mites	8.1	6.0	0.484	3.2	4.0	0.606
Cockroach	4.8	2.0	0.394	3.2	2.0	0.581
Peanuts	1.6	2.0	0.696	1.6	2.0	0.696
Food components (including peanuts)	51.6	38.0	0.150	25.8	18.0	0.324
Pollen	38.7	30.0	0.336	16.1	16.0	0.985
Fungal allergens	19.4	2.0	**0.003**	6.5	0.0	0.090
Domesticated animals	4.8	4.0	0.601	1.6	0.0	0.554
	Sm*+ (n=53)	***Sm*- (n=58)**		***Sm*+ (n=37)**	***Sm*- (n=75)**	
CCD-bearing components	35.9	29.3	0.462	15.1	15.5	0.951
Venoms	60.4	34.5	**0.006**	30.2	13.8	**0.036**
Dust mites	9.4	5.2	0.309	3.8	3.5	0.656
Cockroach	3.8	3.5	0.656	1.9	3.5	0.534
Peanuts	0.0	1.7	0.523	0.0	1.7	0.523
Food components (including peanuts)	49.1	41.4	0.417	20.8	22.4	0.832
Pollen	35.9	32.8	0.732	15.1	15.5	0.951
Fungal allergens	18.9	3.5	**0.009**	5.7	1.7	0.276
Domesticated animals	5.7	3.5	0.457	1.9	0.0	0.477
	Any nematode infection¶ (n=26)	**Uninfected (n=86)**		**Any nematode infection¶ (n=26)**	**Uninfected (n=86)**	
CCD-bearing components	38.5	31.4	0.502	19.2	15.1	0.617
Venoms	57.7	44.2	0.227	23.1	22.1	0.916
Dust mites	7.7	6.9	0.595	0.0	4.7	0.342
Cockroach	3.9	3.5	0.658	3.9	2.3	0.551
Peanuts	3.9	1.2	0.412	3.9	1.2	0.412
Food components (including peanuts)	53.9	43.0	0.332	34.6	18.6	0.086
Pollen	38.5	33.7	0.657	15.4	16.3	0.592
Fungal allergens	23.1	8.1	**0.037**	11.5	1.2	**0.038**
Domesticated animals	0.0	5.8	0.260	0.0	1.2	0.768

Table shows proportions of rural participants (categorised by helminth infection status) that had an ISAC-detectable IgE response or were sensitised (IgE≥0.3 ISU) to at least one allergen component within the larger allergen groupings of CCDs, venoms, dust mites, cockroach, peanut, food, pollen, fungi and domesticated animals. Chi-square / Fisher's exact tests were conducted to assess differences between helminth infected and uninfected participants. These tests were conducted within the framework of a Monte Carlo simulation algorithm based on 1000 permutations in order to adjust for multiple testing.
**Schistosoma mansoni infection determined by Kato-Katz and/or PCR*
¶Infection with any of Ascaris lumbricoides, Trichuris trichiura (assessed by KK), Necator americanus, Strongyloides stercoralis (assessed by PCR) and Mansonella perstans (assessed by modified Knott's method).
***ISAC**: Immuno Solid-phase Allergen Chip; **ISU**: ISAC standardised units*

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CHAPTER 8. SUMMARISING DISCUSSION AND CONCLUSIONS

8.1 Preamble

Each Research Paper in this thesis includes a detailed discussion of the results presented therein. This chapter summarises the main findings in each of the papers, interprets and synthesises these findings, highlights study strengths and limitations, and offers recommendations for future research.

8.2 Summary and interpretation of main findings

8.2.1 Research Paper 1 (Chapter 4)

Research Paper 1 shows prevalence and risk factors for SPT reactivity (to common environmental allergens in the study setting[1]), allergen extract-specific IgE sensitisation, wheeze, rhinitis, urticaria and visible flexural dermatitis, in Ugandan rural and urban settings. Urban-rural differences in prevalence and risk factors for these allergy-related outcomes, and in prevalence of helminth infection, are shown. The rural setting had a significantly higher prevalence of helminth infections, allergen extract-specific IgE sensitisation and urticarial rash, compared to the urban setting. Prevalence of SPT reactivity and visible flexural dermatitis was higher in the urban setting.

Inverse associations between SPT reactivity and 1) the rural setting, 2) helminth infections in the rural setting, are in line with previously published findings that support a protective role (against allergy) for helminths and the rural environment.[2-4] Interestingly in the urban setting, light *S. mansoni* infection was positively associated with SPT reactivity, while moderate and heavy infections were inversely associated with the same outcome. This is not a new phenomenon: protective effects of helminths on allergic effector responses may depend on intensity and chronicity of infection:[5,6] heavy, chronic helminth infections are associated with immunoregulation (with spill-over inhibitory effects on allergic responses), while light and/or acute infections may promote allergy-like responses.[5] This phenomenon may not apply in the rural setting described in this thesis because it is a highly endemic area characterised by heavy infections. Positive

associations between allergen extract-specific IgE and helminth infections and helminth-specific antibodies, in both the rural and the urban survey, may be attributed to protein cross-reactivity and/or carbohydrate cross-reactivity between helminth antigens and allergens (the latter is assessed in Chapters 6 and 7).

Risk factors for allergy-related outcomes (dominated by helminth [*S. mansoni*] infection and helminth-related immunological and epidemiological factors) also differed by setting. Pairwise associations between clinical allergy outcomes, and between atopy and clinical allergy outcomes, were stronger among urban compared to rural participants. Setting (rural versus urban) was an effect modifier for several allergy risk factors; for example, associations between SPT reactivity and *S. mansoni* infection and *Schistosoma*-specific IgG4 were positive in the urban setting and inverse in the rural setting.

Research Paper 1 further investigated the hypothesis that helminth (*S. mansoni*) infections are effect modifiers of associations between risk factors and allergy-related outcomes, and whether they were likely to mediate the differences in prevalence and risk factors for allergy-related outcomes, between the urban and the rural setting. Despite indicative trends, statistical analyses implied that the helminths (specifically *S. mansoni*) are unlikely to be the sole mechanism of the observed effect modification between the low-helminth exposure urban setting and the high-helminth-transmission rural setting. Instead, observations that *S. mansoni* infections were important risk factors for allergy-related outcomes (inversely or otherwise) imply that helminths mainly impact allergy-related outcomes through direct effects, rather than as risk modifiers.

Notably, work conducted by Pinot de Moira and colleagues among Ugandan children[7] showed that hookworm, but not *S. mansoni* infection, dissociated house dust mite-specific IgE from histamine release. This implies that there might be differences in mechanisms between *S. mansoni* and other helminth infections. Analyses conducted in Research Paper 1 did not show a role for hookworm or other helminths; however, the low prevalence of other helminths gave little power to assess any such effects.

8.2.2 Research Paper 2 (Chapter 5)

Research paper 2 reports findings from the LaVIISWA baseline survey, showing that total, SWA-, SEA- and allergen extract-specific IgE and IgG4 responses are positively associated with both *S. mansoni* infection and SPT reactivity. This paper presents results from data collected before community-based anthelminthic intervention was provided as part of the LaVIISWA trial. Further, unpublished results presented in Chapter 5 show that a similar trend was observed after three years of the LaVIISWA trial, as well as in a parallel urban survey. Similar to helminth infection prevalence patterns in the rural and urban surveys, concentrations of total, SWA-, SEA- and allergen extract-specific IgE and IgG4 responses were higher among rural compared to urban survey participants. The observation that allergen extract-specific IgE levels were also higher in the rural survey may be attributed to cross-reactivity between helminth antigens and common environmental allergens in this setting.[8-12]

These antibody responses did not differ between intensive and standard trial arms in the rural survey. The main outcome survey of the LaVIISWA trial also found no evidence of a difference in prevalence of allergy-related disease between intensive and standard trial arms (**Research Paper 7, Appendix 3,** this thesis). Factors potentially contributing to the lack of impact on allergy outcomes are discussed in Research paper 7, and include failure to eliminate helminth infections from villages in both trial arms, and the longstanding immunological effects of helminth exposure in this endemic setting. In the current analysis, persistence of antibodies at pre-intervention levels, and the similarity in antibody profiles between trial arms, may explain lack of differences in clinical allergy outcomes between the trial arms, at least in part.

Although IgG4 responses and SWA- and SEA-specific antibody concentrations were similar between asthmatic schoolchildren and non-asthmatic controls in the asthma case-control study (unpublished results, section 5.4), total and allergen extract-specific

IgE concentrations were higher among asthmatics, providing support to mounting evidence that most asthma in the tropics is atopic, contrary to previous perception.[13,14]

The potential protective role of IgG4 against allergic effector responses may best be assessed relative to IgE. Thesis Chapter 5 shows that total and allergen-specific IgG4/ IgE ratios and total IgE/ allergen-specific IgE ratios were inversely associated with SPT reactivity, wheezing and asthma. These results strengthen the argument that the IgG4–IgE balance and/or the total IgE–allergen-specific IgE balance (both significantly influenced by helminth infection) are important in protection against clinical allergy. Potential mechanisms are discussed in Chapter 1, and include **1)** IgG4-mediated blockage of allergen recognition by IgE (when IgG4 and IgE have similar antigenic specificity[15-17]), **2)** FcγRIIB-dependent IgG4 inhibition of IgE-mediated effector cell activation[17-19] and **3)** saturation of IgE receptors by non-specific, polyclonally-stimulated IgE[20] hence reducing chances that an allergen will result in cross-linking of FcεRI-bound IgE.[8,21]

8.2.3 Research Paper 3 (Chapter 6)

Chapter 6 presents microarray analysis of IgE and IgG responses to core β-1,2-xylosylated and α-1,3-fucosylated N-glycans, showing that responses to these core modified N-glycans were higher in the *S. mansoni*-endemic rural communities compared to lower *S. mansoni* transmission urban communities. Mass spectrometry-based studies,[22-24] have shown that core β-1,2-xylose and core α-1,3-fucose motifs are not only present on glycoproteins of common environmental allergens from plant and invertebrate sources, they are also expressed during specific schistosome life stages (Figure 1.3, Chapter 1). This may explain the urban-rural differences (in reactivity to core β-1,2-xylose and α-1,3-fucose) observed in the current study settings.

Research Paper 3 further shows that associations between *S. mansoni* infection and IgE and IgG reactivity to core modified N-glycans vary by setting. In the urban setting, IgE and IgG responses to both core β-1,2-xylose and core α-1,3-fucose were positively

associated with *S. mansoni* infection. However, in nearby rural communities, IgE and IgG responses to core β-1,2-xylose were strongly positively associated with *S. mansoni* infection while reactivity to core α-1,3-fucose was elevated in both *S. mansoni* infected (*Sm* KK+/PCR+) and uninfected (*Sm* KK-/PCR-) individuals. As discussed in Research Paper 3, these observations may be attributed to the universal *S. mansoni* infection exposure in the rural setting, where 'uninfected' individuals carry light infections not detectable by Kato-Katz and/or PCR. Core α-1,3-fucose motifs can be found on N-glycans from *S. mansoni* eggs, but not on N-glycans from cercariae and adult worms.[22,23,25] It is possible that responses to core α-1,3-fucose persist following active infection in the high *S. mansoni* exposure rural communities. Moreover, it has been shown that eggs and hepatic granulomas stay long after clearance of worms in mice.[26] Also in the rural communities, the concentration of antibodies to core α-1,3-fucose modified N-glycans peaked ahead of the peak of *S. mansoni* infection intensity, while the peak of antibodies to N-glycans with only core β-1,2-xylose coincided with it. These results suggest that reactivity to core β-1,2-xylose is more responsive to change in *S. mansoni* exposure: core β-1,2-xylose is present on cercariae, despite being absent in adult worms.[22,23,27]

The distinctive relationships between *S. mansoni* infection (and intensity) and reactivity to core β-1,2-xylose and α-1,3-fucose modified N-glycans warrant further exploration for associations with protective immunity against *S. mansoni.* In Chapter 7 (discussed below), associations between reactivity to core modified N-glycans and sensitisation to other environmental exposures (other than *S. mansoni*) were explored.

8.2.4 Research Paper 4 (Chapter 7)

Chapter 7 aimed to assess to what extent cross-reactive carbohydrate determinant (CCD)-specific IgE determines helminth-allergy associations in Uganda. The main hypothesis was that elevated IgE responses to specific immunogenic CCD N-glycans during chronic helminth infection might dominate over allergen protein-specific IgE,

resulting in reduced allergic effector responses. The ImmunoCAP® test was employed to measure IgE against house dust mite, cockroach and peanut extracts. The ISAC® microarray provided a component-resolved assessment of IgE reactivity to natural and recombinant allergen components, including those known to carry CCDs. The key features of classical CCDs are N-glycans with core β-1,2-xylose and core α-1,3-fucose substitutions. To further elucidate reactivity to CCDs in Ugandan individuals, a glycan microarray was employed to measure IgE to glycans with and without core β-1,2-xylose and core α-1,3-fucose substitutions.

ImmunoCAP-determined allergen extract-specific IgE sensitisation was higher in the rural compared to the urban survey, and among asthmatics compared to non-asthmatic controls, similar to findings shown in Chapter 4. However, sensitisation to allergen extracts did not reflect sensitisation to their major, established natural and recombinant allergenic components among non-asthmatics and rural and urban participants. Allergen extracts may contain cross-reactive protein and carbohydrate components that are conserved in other environmental antigens such as those from *S. mansoni.*[8] Therefore, sensitisation to established major allergenic components is rare in the study settings (especially among helminth infected, rural individuals), and estimation of true prevalence of allergic sensitisation is complicated by protein and/or carbohydrate cross-reactivity between helminths and allergens.

This postulation is supported by the observation that many participants recognised natural purified CCD-bearing components on the ISAC allergen microarray, suggesting that in this setting, allergen extract-specific IgE may primarily be raised against carbohydrate groups shared with other environmental antigens, such as helminths. Studies have shown that CCDs contribute significantly to cross-sensitisation between *Schistosoma* antigens and peanut, pollen or insect venom allergens.[9,10,28] Here also, glycan array-determined responses to classical CCD epitopes (core β-1,2-xylose and core α-1,3-fucose) were strongly associated with *S. mansoni* infection and allergen

extract-specific IgE. Sensitisation to recombinant insect venom components was also high, especially among *S. mansoni* infected individuals. This may be attributed to sensitisation to Venom-Allergen-Like proteins expressed by schistosomes.[29]

As mentioned before, one of the key differences between the rural and the urban setting was in helminth infection prevalence and intensity. Chapter 7 shows that IgE reactivity to venom protein components, CCD-bearing components (measured by ISAC) and classical CCD N-glycan epitopes (assessed by glycan microarray), was higher among rural participants. Conversely, responses to the major components (predominantly recombinant proteins) from common environmental allergens in this setting,[1] especially house dust mite, were higher in the urban compared to rural setting, and among asthmatics compared to controls. These data imply that in the rural *Sm*-endemic settings, IgE may be less effectively induced against established major allergenic protein components than against CCDs.

Carbohydrate-specific IgG has been hypothesised to block IgE-mediated responses;[9,10,30,31] this thesis further hypothesised a protective role for CCD-specific IgE against allergic effector responses. However, ISAC-determined CCD-specific IgE was similar between asthmatics and non-asthmatic controls, and between SPT positive and negative individuals. Instead, glycan microarray IgE reactivity to core α-1,3-fucosylated N-glycans was inversely associated with asthma. In line with this finding, Do *et al.*[32] recently found that an N-glycan from Bla g 2, a major German cockroach allergen, inhibited basophil histamine release and IL-4 production. A more detailed analysis of this N-glycan revealed that it was core fucosylated. However, Do *et al*. did not show a protective role for N-glycan-specific IgE. Moreover, glycosylated Bla g 2 was a stronger inducer of basophil activation than its de-glycosylated counterpart. These findings, and those reported in this thesis, imply that reactivity to specific glycans or glycan motifs may be important in protection against allergic disease through yet-to-be-defined mechanisms. More experimental studies are required to unravel the potential

mechanisms.

In helminth-endemic settings, it is also important to consider that any observed inverse associations between CCD-specific antibodies and clinical allergy may denote an epiphenomenon, simply reflective of helminth-mediated protection against allergies through other mechanisms. Therefore, studies exploring potential protective roles of CCDs in absence of helminth infection are important. Wilbers and colleagues[33] have recently demonstrated that helminth glycoproteins, including those carrying core β-1,2-xylose and α-1,3-fucose substituted N-glycans, can be reconstructed in plant systems.[33] This provides an important avenue for obtaining tailored carbohydrate epitopes to explore the potential protective role of specific glycans, using animal models and *in vitro* human experiments.

8.3 Thesis strengths

8.3.1 Sample sizes and similar procedures across studies

This PhD thesis presents findings from analyses of data collected from three well-designed large studies on allergy in Uganda. The large sample sizes enabled precise assessment of associations between epidemiological and immunological factors related to helminths and allergy. Where subsets of samples were chosen for analysis, such as for the N-glycan and allergen microarray assays, selections were done in such a way that sample sizes were comparable to or larger than those reported in similar studies.[34-38]

The LaVIISWA trial, the urban survey and the asthma study were all designed specifically to examine allergy-related outcomes, and the methods (and procedures) were designed to be as similar as possible between the studies. This ensured validity of the comparisons (of immunological profiles and other factors) made between the studies.

8.3.2 Methods

Robust methods were used in laboratory and statistical analyses. For example, a component-resolved microarray approach was used to profile allergic responses, complementing the singleplex immunoassays used for allergy testing. Statistical procedures such as PCA and HCA were used in data reduction (and consequently for analysis) of complex allergen and glycan microarray data. For these data, methods used to compute multiplicity corrected p-values ensured that the findings were statistically plausible. Several quality control (QC) measures were also implemented for several laboratory analyses. For example, QC for PCR on stool-derived DNA was conducted by collaborators in the Netherlands (Dr Jaco Verweij, Laboratory for Medical Microbiology and Immunology, St Elisabeth Hospital, Tilburg), glycan microarray analyses were conducted in conjunction with colleagues in the glycobiology department at the Leiden University Medical Centre, and the ImmunoCAP® and the ImmunoCAP® ISAC immunoassays were semi-automated with appropriate internal controls. Appropriate data management and cleaning procedures were also in place, as described in the Methods section 3.8.

8.3.3 New findings

This thesis highlights the potential role of the total IgE – allergen-specific IgE balance and the IgG4 – IgE balance in mitigation of allergic effector responses, providing evidence from large population-based studies. Results showing the potential importance of the IgG4 – IgE balance in protection against clinical allergy build upon previous findings that apportion it a significant role in allergen-specific immunotherapy.[39]

This thesis shows, for the first time, that antibody responses to the classical CCD epitopes (core β-1,2-xylose and α-1,3-fucose) have distinctive relationships with *S. mansoni* infection and intensity, possibly reflecting their different contributions to immunity to *S. mansoni*. This thesis further provides invaluable insights into the potential

protective effect of IgE reactivity to specific CCD epitopes (such as core α-1,3-fucose) against clinical allergy, which has not been assessed before in human participants.

8.4 Thesis limitations

The research papers presented in this thesis report a large number of statistical tests, increasing the possibility of chance findings. In Chapters 4 and 5, no formal adjustment for multiple testing was done, instead the focus was placed on biological credibility in reference to published works, as well as on consistency of findings and trends of associations between exposures and outcomes. In Chapters 6 and 7, correction for multiple testing was done by conducting permutation tests using a Monte Carlo simulation approach.

A large proportion of the data used in this thesis was obtained from cross-sectional surveys; so, assessment of temporality was not possible. Therefore, it was difficult to establish causality when assessing helminth-allergy associations. The LaVIISWA trial was originally designed to address this; however, intensive versus standard anthelminthic treatment had no effect on most primary study outcomes. Nonetheless, important deductions can be made from this thesis' findings, owing to the strong patterns of associations observed.

As discussed in **Research Paper 1** (Chapter 4), rural communities had received three years of mass anthelminthic treatment at the time of the allergy outcomes survey, while the urban community had considerable exposure to light helminth infections. However, the two settings were still quite distinct, as helminth prevalence remained high in the rural setting (presumably due to high re-infection rates), providing an interesting sample for assessing the impact of urban-rural differences in helminth infection exposure on allergy related outcomes.

In the rural population, exposure to helminths was universal, and there were no data on duration of infection. This is a potential limitation in assessment of helminth-allergy associations and underlying mechanisms. Therefore, comparisons with the low helminth

exposure urban setting were important in assessment of plausibility of deductions derived from analysis of helminth-allergy associations in the rural setting.

8.5 Conclusion(s)

This thesis presents an immuno-epidemiological description of antibody profiles associated with helminth infection, allergic sensitisation and clinical allergy in rural and urban Uganda, providing important insight into mechanisms underlying the complex helminth-allergy associations in tropical low-income countries.

The major conclusions from this PhD research are

1. Despite suggestive trends, there was no statistical proof that helminth (*S. mansoni*) infection mediates urban-rural differences in prevalence and risk factors for allergy-related outcomes in Uganda, implying that helminths are not the sole mechanism. Other environmental exposures are likely to play a role. The observation that *S. mansoni* infection was an important risk factor for allergy-related outcomes (inversely or otherwise) implies that helminths mainly impact allergy-related outcomes through direct effects, rather than as risk modifiers.
2. In communities exposed to helminth infections, the IgG4 – IgE balance and the total IgE – allergen-specific IgE balance may be important in protection against clinical allergy.
3. In tropical settings, helminth-induced CCD-specific antibody responses (specifically IgE) obscure readouts for assessment of atopy.
4. Inverse associations between asthma and IgE to α-1,3-fucose substituted N-glycans imply that antibodies against specific CCD epitopes may be important in protection against clinical allergy, through yet-to-be-defined mechanisms.

8.6 Antibody-mediated mechanisms of helminth-allergy associations: future perspectives

Chapter 5 highlights the potential importance of the IgG4 – IgE balance in protection against clinical allergy. Immunoglobulin G4 has been implicated in IgG4-related

disease.[40] Despite this, immunoregulation during chronic helminth infection appears to be a more prominent role for IgG4,[15] as well as induction of allergen tolerance during specific immunotherapy.[39] Besides, IgG4 does not activate the complement system or lead to formation of immune complexes, and is not associated with mast cell or basophil degranulation.[41] Its major mode of action against inflammation seems to be blockage of IgE-mediated processes. This thesis offers solid support for the regulatory role of IgG4. Further experimental animal studies would be ideal for studying the protective role of IgG4; however, they are hampered by the absence of IgG4 in mice. Therefore, *in vitro* human experiments should be the focus of future work. For example, by using a high-throughput microarray screening platform, helminth antigens that induce strong serum IgG4 responses and little or no IgE may be identified. Such antigens may be important for therapeutic strategies against clinical allergy. For example, IgG4 induced by such antigens may trigger FcγRIIB-dependent inhibition of IgE-effector cell degranulation.[17-19]

Immune responses to some helminths are shaped to a great extent by the glycome and this has been clearly demonstrated for schistosomes (in this thesis and elsewhere). Responses to carbohydrate antigens may be part of the worm's strategy to protect itself from IgE-mediated attack; however, they may also contribute to protective immunity against helminth infection, or could be useful in diagnostics, but detailed research is needed to determine their role and potential applications. Furthermore, profiling of helminth CCD-specific responses may improve current understanding of potential mechanisms of protection against allergy. Before work conducted as part of this PhD research, these responses were not yet well characterised at a population level. Chapter 6 highlights rural-urban differences in IgE and IgG responses to key components of the schistosome glycome (core β-1,2-xylose and α-1,3-fucose substituted N-glycans) and shows that responses to these components have distinctive relationships with *S. mansoni* infection and intensity. It has been previously implied that responses to cross-reactive carbohydrates have the potential to inhibit clinical peanut allergy;[28] Although

results presented in Chapter 7 of this thesis showed no overall difference in CCD-specific IgE between participants with and without SPT positivity or asthma, there were inverse associations between reactivity to core α-1,3-fucose substituted N-glycans and asthma, signifying potential for specific CCD epitopes in protection against asthma. Definitive proof of CCD-mediated protection against clinical allergy is required and mechanistic studies in animal models, and using human samples in *in vitro* experiments, are warranted.

Overall, findings presented in this thesis provide important insight into mechanisms underlying the complex epidemiological helminth-allergy trends in Uganda. This is important for approaches aimed at reducing the impact of the on-going epidemiological transition on allergy prevalence in the tropics and for therapeutic strategies against allergy-related diseases.

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APPENDICES

9.1 APPENDIX 1: Research Paper 5: A life without worms

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PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	GYAVIIRA NKURUNUNGI
Principal Supervisor	ALISON ELLIOTT
Thesis Title	HELMINTH-ALLERGY ASSOCIATIONS IN RURAL AND URBAN UGANDA: INSIGHTS FROM ANTIBODY STUDIES

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE		
When was the work published?	18 March 2017		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I participated in carrying out literature searches, and wrote the section on helminth manipulation of host systems. I also critically revised all other sections of the manuscript.

Student Signature: Ahmugi **Date:** 14th FEB 2019

Supervisor Signature: _____ Date: 15/2/2019

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REVIEW

A life without worms

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Worms have co-evolved with humans over millions of years. To survive, they manipulate host systems by modulating immune responses so that they cause (in the majority of hosts) relatively subtle harm. Anthelminthic treatment has been promoted as a measure for averting worm specific pathology and to mitigate subtle morbidities which may include effects on anaemia, growth, cognitive function and economic activity. With our changing environment marked by rapid population growth, urbanisation, better hygiene practices and anthelminthic treatment, there has been a decline in worm infections and other infectious diseases and a rise in non-communicable diseases such as allergy, diabetes and cardiovascular disease. This review reflects upon our age-old interaction with worms, and the broader ramifications of life without worms for vaccine responses and susceptibility to other infections, and for allergy-related and metabolic disease. We touch upon the controversy around the benefits of mass drug administration for the more-subtle morbidities that have been associated with worm infections and then focus our attention on broader, additional aspects of life without worms, which may be either beneficial or detrimental.

Keywords: Allergy, Anthelminthic, Helminths, Infectious diseases, Metabolic disease, Vaccines

Introduction

Over a billion people are estimated to be infected with helminths, most living in areas of poverty.[1,2] Helminths have co-existed with mammals for millions of years. Their lifecycles have evolved to ensure their survival while minimising harm to the mammalian host. Soil transmitted helminths (STH) such as hookworm, *Ascaris lumbricoides, Trichuris trichiura* and *Strongyloides stercoralis* spend part of their lifecycle in soil and gain access to their human host through skin penetration or ingestion. For the filarial nematodes, an insect vector takes up microfilariae during a blood meal and, after development in the insect, the parasite is injected into the next host during another blood meal. For water borne helminths such as *Schistosoma*, cercariae shed by the snail intermediate host access the definitive human host by skin penetration during contact with infested water. After migratory and development stages, adult worms lodge in body tissues such as the gut, blood vessels and lymphatics. In most cases, helminths do not replicate in the mammalian host.[3]

Helminths induce short and long term morbidity, and pathology in some body systems: gastrointestinal tract (malabsorption, diarrhoea, macro and micronutrient deficiencies, bleeding, intestinal obstruction, rectal prolapse), liver (peri-portal fibrosis, cholangitis, cholangiocarcinoma, hepatocellular carcinoma), cardiovascular system (anaemia), lymphatic system (lymphoedema), central nervous system (blindness, epilepsy), genito-urinary tract (haematuria, hydronephrosis, bladder cancer), lungs (Loeffler's syndrome).[4] These effects depend on the type and number of helminths in the host. Treatment is deserved to avert these harmful effects.

Societies in developing countries are experiencing remarkable population growth, urbanisation and lifestyle changes. With better hygiene and 'deworming' interventions, helminth infections are declining. Concurrently, there is a rise in non-communicable diseases (NCDs) such as diabetes[5] and cardiovascular diseases,[6] contributing significantly to global mortality and attributed largely to changes in diet and lifestyle. Could the decline in helminth infections be playing a role in this epidemiological transition? With helminth elimination the ultimate goal of mass drug administration (MDA) programmes,[7] it is of interest to reflect on the prospect of a worm-free life. Do we clearly understand, are we ready for, the consequences of life divorced from the partnership established over millions of years? In this narrative review we discuss current evidence regarding the benefits of MDA, ways in which worms manipulate us, and the possible

effects of helminth infection on responses to vaccines and unrelated infectious diseases, and on allergy and metabolic disease.

How much do we benefit from MDA?

MDA entails administration of anthelminthic medicines without reference to an individual's infection status, or test of cure. The World Health Assembly endorsed MDA for school children as a schistosomiasis and STH control strategy for high transmission settings[8] and this has been widely adopted.

MDA policy is premised on anticipated benefits for helminth-specific pathology, maternal anaemia, birth weight, childhood growth, anaemia, cognitive function, school performance and long term economic returns. We do not question the benefit of MDA for controlling pathologies such as schistosome-induced fibrosis, hookworm-induced anaemia, elephantiasis and river blindness. However, the impact of MDA on more subtle morbidities associated with helminths has been difficult to demonstrate.

Mass treatment for hookworm in the American south at the turn of 20th century was associated with greater school enrolment, attendance and literacy and long-term gain in income.[9] Further, in 2004, Miguel and Kremmer published a highly influential report showing an association between school-based MDA and reduced school absenteeism among Kenyan children.[10] Ten years later, these children who were dewormed at school had more years of school enrolment, more time in employment and longer work hours each week.[11] However, recent reanalyses of the original data have highlighted the challenges of evaluating such interventions.[12,13]

A large cluster-randomised trial in India with one million pre-school children showed little effect of regular deworming on mortality in pre-school children.[14] A Cochrane review[15] concluded that treating children known to have STH may improve weight gain but evidence of benefits on haemoglobin, school attendance and cognitive function is limited; also that community based treatment programmes had little or no effect on these outcomes. Similarly, a systematic review found the evidence insufficient to link helminths to cognitive performance[16] and a further meta-analysis concluded that mass deworming of children had little or no effect on weight, height, cognition, school attendance or mortality.[17]

WHO recommends anthelminthic treatment during pregnancy, hoping that it will reduce maternal anaemia, increase birth weight and reduce mortality. The benefits are not yet clear.[18] We, and others, have found limited overall effects[19] of anthelminthic use during pregnancy on maternal anaemia, and none on birth weight, perinatal mortality or congenital abnormalities.[20–23] Anthelminthic treatment during pregnancy did not affect infectious disease incidence or response to immunisation.[24,25] A Cochrane review notes that evidence is insufficient to recommend use of anthelminthic medication for pregnant women in the first trimester and administration of a single dose anthelminthic was not associated with any impact on maternal anaemia.[26]

There has been debate on the policy of MDA[27] and systematic review methodology has been questioned in its application to helminths.[28,29] However, it brings to light the need for more evidence to support MDA and to understand fully its benefits.

How do worms manipulate us?

The age-old colonisation of mammals by helminths has been successful mainly because of the latter's shrewd manipulation of host systems (Figure 1).

Helminths employ enzymes and other excretory/secretory proteins to disrupt and alter host tissues, thus successfully migrating, feeding, establishing niches and developing strategies to exit the host to complete their life-cycles. For example, *Schistosoma* cercariae contain proteases that aid in skin penetration[30] while the major excretory/secretory protein of *Trichuris trichiura* induces pore formation to facilitate helminth entrenchment in the gut.[31]

Loss of mucosal and epithelial integrity as helminths take root in the host is often accompanied by release of inflammatory mediators, such as alarmins and damage associated molecular patterns,[32] normally detrimental to the helminth and its host. However, helminth excretory/secretory products also work to offset this. For example, secreted products of *Heligmosomoides polygyrus* block production of the alarmin IL-33, a key inducer of Th2-type inflammation.[33] Besides, to curb host morbidity from helminth-inflicted tissue injury, helminth-induced mediators spearhead tissue healing and remodelling.[34]

But perhaps the helminth's most potent survival weapon is the wide array of mechanisms developed to evade or regulate a vigilant host immune system. At the helm are helminth-induced Th2-type and regulatory immune responses. Helminth-induced Th2 cytokines interleukin (IL)-4 and IL-13 promote alternative activation of macrophages, resulting in production of large amounts of immunomodulatory IL-10 and transforming growth factor (TGF)-β,[35] and T cell hypo-responsiveness involving regulatory T cells.[36] Helminth-induced, IL-10-producing 'regulatory B cells' have also been demonstrated.[37] A notable consequence of helminth-induced immunomodulation is the attenuation of responses to 'bystander' antigens,[38] widely implicated in the helminth-associated modulation of immune responses to a number of non-communicable and communicable diseases.

Helminth-allergy interactions are a good example of the bystander effect. Although antigenic targets for allergen- and helminth-specific immune responses are similar,[39] helminth infections seem to be protective against allergy-related conditions in both humans and mice. Current evidence[32,40] points to an extensive array of immunomodulatory mechanisms underlying inverse helminth-allergy associations. They range from induction of IL-10-producing regulatory T cells, regulatory B cells and alternatively activated antigen presenting cells, promotion of polyclonal IgE synthesis and immunoglobulin class switching to IgG4, to suppression of release of alarmins (such as IL-33) and inhibition of type 2 innate lymphoid cell activity.

Host metabolic responses may also be influenced by helminth infections. For example, *S. mansoni* egg antigen-treated obese mice have increased levels of white adipose tissue Th2-type cells, modified macrophage activation and reduced adipose tissue mass and improved insulin sensitivity.[41] Non-obese diabetic mice infected with *H. polygyrus* and *Trichinella spiralis* are protected against type-1 diabetes through the Th2-associated reduction of inflammatory autoimmune responses.[42] There is also recent evidence in humans and mice that helminths may protect against inflammatory

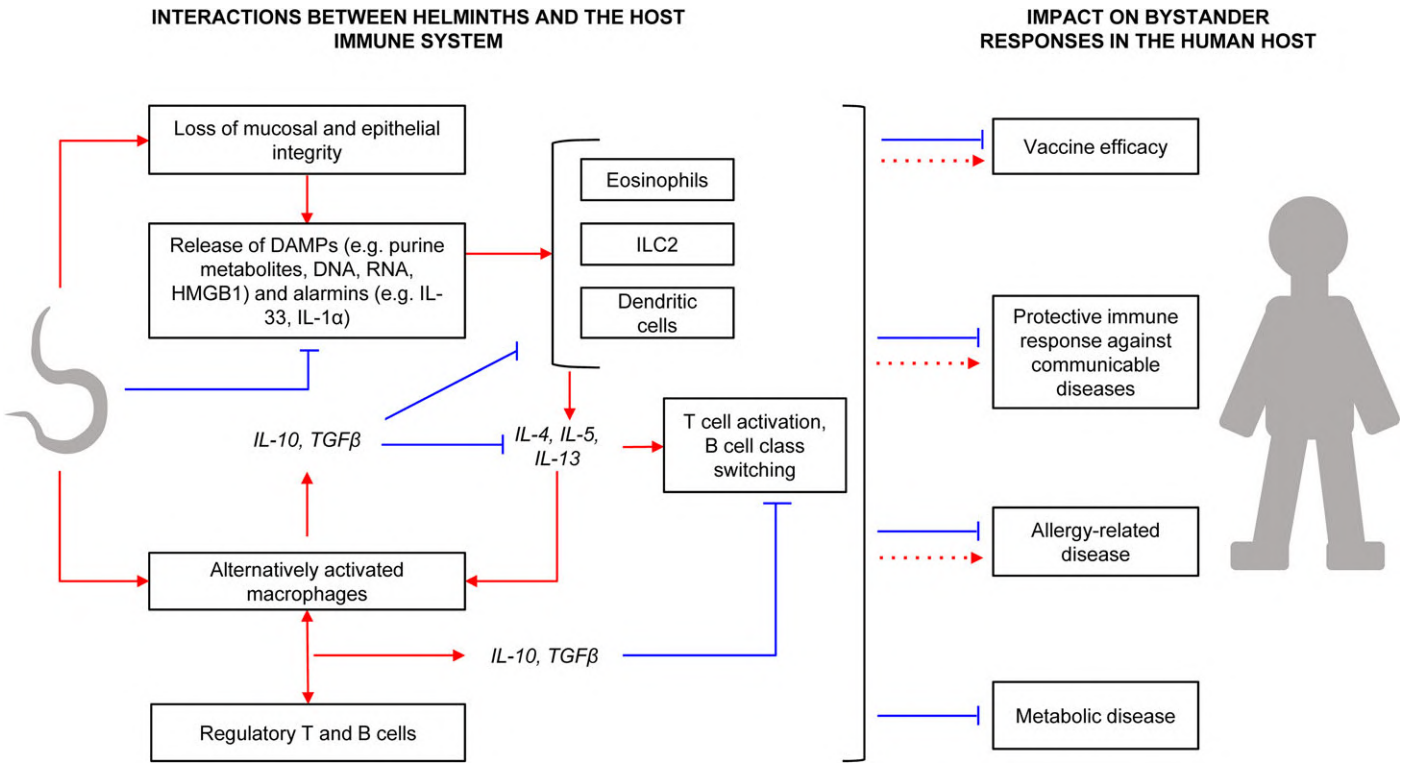

Figure 1. Interactions between helminths and the host immune system, and the impact on bystander responses. Red arrows and blue lines denote positive and suppressive effects, respectively. Helminth migration in the host results in tissue injury, resulting in release of Damage Associated Molecular Patterns (DAMPs) and alarmins. DAMPs and alarmins are involved in the initial activation of eosinophils, type 2 innate lymphoid cells (ILC2) and antigen presenting cells (APCs) such as dendritic cells (DCs), which then mediate further inflammation in the host. However, some helminth secretory products can suppress alarmin release and DC maturation, and some helminth enzymes degrade DAMPs. Helminths also interfere with APC activities, promoting an alternative activation phenotype, which results in production of large amounts of IL-10 and TGFβ. These cytokines downmodulate eosinophil, ILC2 and DC responses, and promote lymphocyte hyporesponsiveness involving regulatory lymphocytes. Helminth interaction with host immunity has spillover effects on responses to bystander antigens. For instance, helminth infections may result in impaired immune responses to vaccines and communicable diseases, although specific helminth molecules may actually have enhancing effects. Likewise, there is evidence for both inverse and positive helminth-allergy associations, although any notable effects on metabolic conditions have been beneficial. DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; HMGB1: High Mobility Group Box 1.

bowel diseases through Th2-type immunity-mediated expansion of a protective microbiota.[43]

Helminth-induced bystander response suppression is a double-edged sword. We may benefit from helminth-driven regulation of non-communicable diseases, as elaborated above. However, helminth excretory/secretory products and Th2 cytokines have been shown to suppress anti-microbial functions of innate immune cells (dendritic cells and macrophages), leading to increased differentiation of regulatory T cells and Th2 cells while impeding the development of protective Th1-type responses and potentially compromising immunity to several communicable diseases.

Worms and vaccines

Following recognition of the Th1/Th2 hypothesis,[44] the contrasting ability of mycobacterial and helminth antigens to elicit Th1 and Th2 responses, respectively, and mutual inhibition between these opposing effects,[45] it was proposed that helminth co-infection might account for the poor efficacy of vaccines such as BCG in tropical settings and the high prevalence of TB and HIV in Africa.[46] As helminth prevalence declines, will vaccines become more effective, and susceptibility to other infectious diseases decrease?

Studies in animal models largely suggest that this will be the case. In the mouse, infection with *H. polygyrus* (a nematode with a life-cycle confined to the gut) modified the response to a malaria protein vaccine, resulting in reduced antibody and Th1 responses, increased Th2 and regulatory responses and impaired protection against malaria challenge.[47] Treatment of the helminth before, but not after, immunisation abrogated these effects, emphasising the importance of co-infection at the time of immunisation. Similar effects of *H. polygyrus* have been reported for a DNA malaria vaccine, but not for live, irradiated sporozoites,[48] or for live BCG,[49] indicating that the impact of a particular helminth differs by vaccine type: protein, DNA or live attenuated organisms. Mice infected with *Schistosoma* species (which cause systemic infections) show impaired induction of protective immunity both to malaria[50] and to TB challenge (following BCG),[51] indicating that different helminth infections have different effects. *Schistosoma* infections also resulted in

impaired antibody responses to toxoid and protein vaccines—but a study on hepatitis B immunisation showed a gradual recovery of the response when the infection was treated after immunisation.[52] The life-cycle of *Trichinella spiralis* involves an intestinal phase, followed by encystment in skeletal muscle; suppression of the IgA response to cholera toxin[53] and to hepatitis B immunisation[54] has been demonstrated during the intestinal, but not the muscle, stages of the life-cycle. While these experiments demonstrate suppressive effects, intraperitoneal injection of *Ascaris* extract concurrently with BCG has been shown to enhance macrophage activation and suppress BCG replication[55] and a protein from the filarial worm *Onchocerca volvulus* shows promise as an adjuvant for influenza vaccine.[56] Together, studies in mice show that helminth infections have important potential to supress vaccine responses, but that helminth species, stage of life-cycle, timing of helminth exposure and treatment, and characteristics of the vaccine may be important determinants of the outcome and that specific helminth molecules may actually have enhancing effects. As well, differences between murine models are likely to result from genetics of the host and intensity of helminth infection used.

In humans, studies of the impact of helminth co-infection on vaccine responses are important in their own right, and also offer an important surrogate for studies on susceptibility to infections, which are much more difficult to undertake. The bystander modulatory effects of chronic helminth infections are of potential direct significance in adolescents and adults when primary or recall immunisation occurs in this age group. For example, for human papilloma virus immunisation, tetanus and other boosters, and during outbreaks, such as the recent Yellow Fever and Ebola epidemics; also when novel vaccines are undergoing initial evaluations in older populations. Observational studies among children and adults have shown associations between helminth infection and suppression of antibody and Th1 responses, particularly during systemic filarial infections and schistosomiasis: vaccines affected include BCG, tetanus, typhoid and a candidate malaria vaccine.[57–65] Hepatitis B immunisation may also be impaired in the context of schistosomiasis[66] but effects may be limited to those with hepatosplenic disease, calling into question the causal mechanisms involved.[67] Clinical trials may help us to test whether helminth induced immunomodulation is causal in suppression of vaccine responses and, so far, these have been confined to effects of geohelminths. Treatment of geohelminths with albendazole has been shown to improve the Th1 response to BCG,[68,69] and the antibody and Th1 response to oral cholera vaccine.[70,71] No studies have yet investigated the effects of treating schistosomiasis or filariases but, on balance, the data so far suggest that vaccine responses will improve with the elimination of worms.

However, the majority of vaccines in current use target pathogens that cause substantial disease and death in early life. They are administered to the very youngest age groups in whom chronic helminth infections have yet to establish themselves. In these age groups, it is maternal infection status that is potentially of greatest importance in terms of impacting on a newborn's capacity for induction of vaccine-specific responses. Evidence that the human fetus could be sensitised in utero to helminths and mycobacterial antigen[72] suggested that prenatal exposure might influence infant vaccine responses. Indeed, initial studies by Malhotra and colleagues showed an association between sensitisation to *Schistosoma* or filarial antigens in utero and a Th2 bias to the infant response to BCG immunisation.[73] Malhotra and colleagues also described adverse associations between prenatal exposure to hookworm and other helminths and the response to diphtheria toxoid and *Haemophilus influenzae* type B (HiB) immunisation in infancy,[74] but this has not been confirmed by results from Uganda where the only association observed was a possible enhancement of IgG responses to pertussis toxin, HiB and hepatitis B among infants of mothers with *Strongyloides*.[25,75,76] A study in Ecuador also showed no association between exposure to maternal geohelminths and infant responses to diphtheria toxoid, tetanus, pertussis, measles, Rubella or HiB, but enhanced IgA responses to polio and rotavirus.[77] An important consideration is that the infant outcome may vary depending on the nature and timing of the exposure to parasite antigens: Malhotra and colleagues showed that infant DT responses were enhanced if the infant was sensitised to malaria antigens, but suppressed if the infant was 'tolerised'.[74] Only one substantive trial has investigated the effects of treating helminths during pregnancy on infant vaccine responses: this did not confirm findings from an earlier pilot[78] and gave only weak evidence of an effect of treating maternal hookworm on the infant response to tetanus or BCG immunisation.[24,25] Further work is needed to understand whether helminth elimination among pregnant women will alter the infant response to key vaccines.

Given the complex effects of helminths on vaccine responses it is not surprising that effects on infectious disease susceptibility are complex too (reviewed elsewhere).[79–81] A possible unifying hypothesis, supported by recent evidence from mouse models,[82] is that chronic helminth co-infection has little effect on the innate response to incident infections (and may even enhance it) but does impair adaptive responses that control replication of established infections. For example, in the case of TB, a recent trial on effects of anthelminthic treatment on bovine TB among wild buffalo in South Africa's Kruger National Park found that regular anthelminthic treatment had no impact on *Mycobacterium bovis* infection incidence, but resulted in lower mortality among *M. bovis* infected animals.[83] Similarly, we found little evidence that helminth co-infection affects susceptibility to TB infection in humans,[84] but recent results suggest that treatment of helminths may abrogate regulatory T cells-mediated suppression of Th1 cell frequency and function in helminth-TB co-infection[85] and hints at improved clinical outcome.[86]

Worms and allergy-related disease

Results from epidemiological studies on the relationship between helminths and allergy have been inconsistent. As for vaccine studies, different helminth species interact with the host's immune system differently, resulting in different clinical outcomes. An earlier review and meta-analysis[87] found that hookworm had an inverse association with asthma (summary odds ratio [OR] 0.50, 95% CI 0.28–0.90), with a 'dose-response' by infection intensity, *A. lumbricoides* showed a positive association and *T. trichiura* showed no relationship. Another meta-analysis[88] showed an inverse association between helminthic

infections and allergen skin sensitisation (summary OR 0.69, 95% CI 0.6–0.79).

Exposure to helminth infections in-utero and in early childhood is negatively associated with allergy risk in childhood. Our birth cohort in Uganda showed that maternal hookworm during pregnancy was associated with a reduced incidence of eczema in childhood (adjusted hazard ration [aHR] 0.71, 95% CI 0.51–0.99), with a dose-response, and that early childhood infections with *T. trichiura* and hookworm were associated with a reduced incidence of childhood eczema.[89] Treatment of maternal helminths during pregnancy increased the incidence of eczema in childhood.[24,90] A study in Brazil also showed that early childhood infections with *T. trichiura* and *A. lumbricoides* were associated with a lower prevalence of allergen skin reactivity in later childhood.[91] In Gabon, a lower prevalence of skin reactivity to house dust mite was reported among children infected with *Schistosoma haematobium* compared to those without the infection.[92] Most studies have considered helminths as an independent variable in regression models, but there is increasing evidence that helminths are effect-modifiers of the relationship between atopy and clinical allergy. We found that maternal hookworm during pregnancy attenuated the association between *Dermatophagoides*-specific IgE and eczema in childhood, as well as the effects of other known risk-factors for eczema such as mother's history of eczema and female gender.[89] This effect-modification has also been reported in studies in Ecuador.[93, 94] A study conducted in Uganda found a positive association between *Dermatophagoides*-specific IgE and histamine release among children without hookworm but not amongst children with hookworm.[95]

Despite the inconsistencies outlined, epidemiological studies have consistently shown a lower prevalence of clinical allergy (and sometimes atopy) in rural compared to urban areas in low and middle income countries.[96–98] This is consistent with the observed low prevalence of asthma/allergy among children raised on farms compared to city dwellers in high income countries.[97] In the high income countries, this farm effect has been attributed to exposure to diverse microbiome on the farm and to the consumption of unpasteurised dairy products.[98] For low and middle income countries, the protective effect had been attributed partly to geohelminths,[93] but the possible role of the microbiome has not yet been extensively explored. Animal studies have demonstrated interactions between helminths and microbiota.[99] Could the microbiome in rural settings explain why, in Ugandan island communities, we found a very low prevalence of clinical allergies, despite positive associations between helminths and reported wheeze (and atopy)?[100]

Additionally, there is increasing evidence of attenuation of the relationship between atopy and allergy among children in rural compared to urban areas in low and middle income countries.[93,101,102] This has been attributed partly to geohelminths, but the role of other infections and microbiome deserves investigation.

Studies on immigrants from rural to urban setting represent natural experiments. One such study[103] found that immigrants from rural Ethiopia to Israel had a low prevalence of atopy and allergy, and a negative association between helminth infection and atopy on arrival, which was quickly reversed after a year of living in Israel. This was attributed to the treatment of helminths, a decline in helminths among the untreated, and exposure to a novel environment.

The helminth-allergy relationship is complicated by the many inter-related factors at play. To obtain a conclusive stand, we need to conduct comprehensive studies that take into account the various helminth-related variables, and the potential interaction and confounding with the microbiome, other infections (such as malaria) and interaction and other environmental exposures. This will require extensive data collection and advanced statistical analyses. But the potential benefits are worth it, for we will be able to understand better how to harness the beneficial effects of worms or the rural environment for the primary, secondary and tertiary prevention of asthma, allergies and other chronic inflammatory conditions that may be associated with a life without worms.

Worms and metabolic disease

A recent systematic review showed that individuals with a previous or current helminth infection were 50% less likely to have metabolic dysfunction.[104]

In diet induced obese mice, chronic infection with *Schistosoma mansoni* lowered whole body insulin resistance and glucose intolerance and improved peripheral glucose uptake and insulin sensitivity. Injection of schistosome antigens induced a similar effect[41] and, in a separate study, reduced atherosclerosis in mice.[105] Mice infected with *H. polygyrus* had lower blood glucose, insulin resistance, fat accumulation than uninfected mice[106] and benefits were sustained even after clearance of the helminth.[107] *Nippostrongylus brasiliensis* infection was associated with decreased weight gain and improved glucose metabolism.[108] Similarly, diet induced obese mice infected with *Litomosoides sigmodontis* or exposed to its antigen had improved glucose tolerance.[109]

In humans, a cross-sectional study in rural China showed that individuals with a history of schistosomiasis infection exhibited lower fasting blood glucose levels compared to controls who had never had schistosomiasis.[110] A study in India[111] reported a lower prevalence of filarial infections in patients with type 2 diabetes than in non-diabetic controls. Patients with type 2 diabetes and lymphatic filariasis had lower concentrations of pro-inflammatory cytokines—IL-6 and GM-CSF—than patients without lymphatic filariasis. Among aboriginal adults in Australia, prior *Strongyloides stercoralis* infection was associated with reduced type 2 diabetes risk.[112] Infection with STH has also been associated with decreased insulin resistance and lower body mass index, abdominal obesity, and lipid levels.[113,114]

Together, these recent findings indicate that helminth infections may convey important benefits for metabolic disease in humans. If so, understanding the mechanisms with a view to harnessing this knowledge for prevention and therapy of metabolic disease is important.

Conclusions

Helminths can be damaging, especially when there are intense infections: therefore, control is good. Some authors have also argued that MDA is a cost-effective health investment for

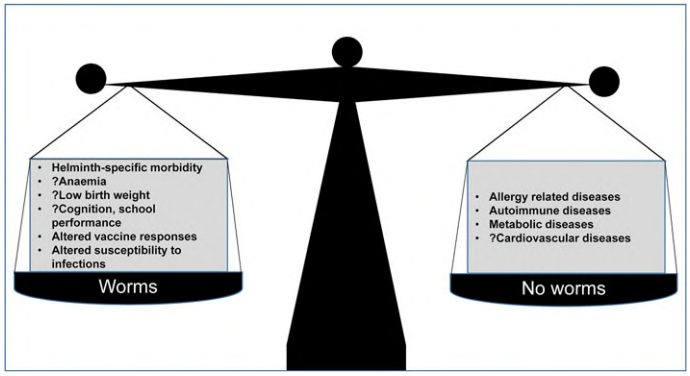

Figure 2. Is 'de-worming' good for us?

governments[115] although, as we have discussed, controlled trials to date have struggled to confirm a major impact of MDA on the subtle morbidities and mortality associated with worm infections in observational studies. As the debate on MDA continues, we need to note that removal of helminths leaves the immune system out of balance. We postulate that helminth elimination will result in a broad array of additional effects, both beneficial and detrimental to human health. The consequences may include altered responses to vaccines and to infectious diseases, and increased susceptibility to inflammatory conditions such as allergy-related disease and metabolic disease (Figure 2). Further work is needed to understand helminth-human interactions and their mechanisms, so that we can mitigate adverse consequences in the event that helminth infections in humans are eliminated.

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9.2 APPENDIX 2: Research Paper 6: Helminths are positively associated with atopy and wheeze in Ugandan fishing communities: results from a cross-sectional survey

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Student	GYAVIIRA NKURUNUNGI
Principal Supervisor	ALISON ELLIOTT
Thesis Title	HELMINTH-ALLERGY ASSOCIATIONS IN RURAL AND URBAN UGANDA: INSIGHTS FROM ANTIBODY STUDIES

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I participated in establishing and conducting laboratory assays and in writing the manuscript

Student Signature: [redacted] **Date:** 14th FEB 2019

Supervisor Signature: [redacted] Date: 15/2/2019

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ORIGINAL ARTICLE EPIDEMIOLOGY AND GENETICS

Helminths are positively associated with atopy and wheeze in Ugandan fishing communities: results from a cross-sectional survey

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allergy; atopy; helminths; household survey; wheeze.

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Abstract

Background: Parasitic helminths are potent immunomodulators and chronic infections may protect against allergy-related disease and atopy. We conducted a cross-sectional survey to test the hypothesis that in heavily helminth-exposed fishing villages on Lake Victoria, Uganda, helminth infections would be inversely associated with allergy-related conditions.

Methods: A household survey was conducted as baseline to an anthelminthic intervention trial. Outcomes were reported wheeze in last year, atopy assessed both by skin prick test (SPT) and by the measurement of allergen-specific IgE to dust mites and cockroach in plasma. Helminth infections were ascertained by stool, urine and haemoparasitology. Associations were examined using multivariable regression.

Results: Two thousand three hundred and sixteen individuals were surveyed. Prevalence of reported wheeze was 2% in under-fives and 5% in participants ≥5 years; 19% had a positive SPT; median *Dermatophagoides*-specific IgE and cockroach-specific IgE were 1440 and 220 ng/ml, respectively. *S. mansoni*, *N. americanus*, *S. stercoralis*, *T. trichiura*, *M. perstans* and *A. lumbricoides* prevalence was estimated as 51%, 22%, 12%, 10%, 2% and 1%, respectively. *S. mansoni* was positively associated with *Dermatophagoides*-specific IgE [adjusted geometric mean ratio (aGMR) (95% confidence interval) 1.64 (1.23, 2.18)]; *T. trichiura* with SPT [adjusted odds ratio (aOR) 2.08 (1.38, 3.15)]; *M. perstans* with cockroach-specific IgE [aGMR 2.37 (1.39, 4.06)], *A. lumbricoides* with wheeze in participants ≥5 years [aOR 6.36 (1.10, 36.63)] and with *Dermatophagoides*-specific IgE [aGMR 2.34 (1.11, 4.95)]. No inverse associations were observed.

Conclusions: Contrary to our hypothesis, we found little evidence of an inverse relationship between helminths and allergy-related outcomes, but strong evidence that individuals with certain helminths were more prone to atopy in this setting.

Allergy-related diseases increased dramatically in affluent and middle-income countries during the twentieth century (1, 2): asthma now affects about 300 million people (3), and eczema 5–20% of children (4). Although these conditions remain relatively rare in low-income countries (LICs), they appear to be increasing (5, 6). It has been hypothesized that helminth infections, still highly prevalent in LICs (7), may contribute to these geographic differences. Globally, the majority of asthma and eczema cases are associated with allergen sensitization or atopy. However, in LICs, the role of atopy in allergy-related disease is less certain (8, 9).

Parasitic helminths evolved to coexist with their mammalian hosts, are often asymptomatic and cause limited mortality. To this end, they have developed mechanisms for

evading or modulating the host immune response. Evidence from animal models, and from *in vitro* studies on human samples, suggests that helminths can modulate the immune response not only to themselves, but also to unrelated pathogens, antigens and allergens (10, 11). Helminths contain a range of molecules homologous to known allergens, but absent from mammals or markedly different from mammalian homologues. These induce IgE responses in mammalian hosts, and there is a strong evidence that this pathway is involved in protective immunity against helminths (12). Modulation of this atopic pathway is likely, therefore, to be particularly important for helminth survival, while concomitantly protecting against allergic disease (13).

Associations between helminths and allergy-related conditions have been investigated in a variety of settings [reviewed in (14–16)]. The majority of studies investigating helminth–atopy associations have either reported an inverse association or no evidence of association. However, results for allergy-related diseases such as asthma and eczema have been less consistent, varying both within and across helminth species. Meta-analyses have indicated that for asthma, *Ascaris lumbricoides* infection is positively associated and hookworm inversely associated, with borderline evidence of a positive association for *Trichuris trichiura* and no association for *Strongyloides stercoralis* (15). Very few studies have investigated associations with schistosomiasis, although those that have generally report an inverse association with atopy (17–20).

Helminths may have an important role as modifiers of associations between markers of atopy and allergy-related diseases. It has been reported that IgE and SPT responses to house dust mite are positively associated in the absence of hookworm but unrelated among hookworm-infected individuals (21). Similarly, studies have found that positive associations between atopy and wheeze, and between atopy and eczema, exist only among individuals who do not have hookworm and not among those infected with hookworm (22, 23). Finally, it has been reported that atopy and wheeze are only positively associated in the absence of concurrent *Ascaris* infection and not in its presence (24).

Intervention studies are an important tool for understanding the relationship between helminths and allergy-related diseases; however, findings of previous studies on the impact of anthelminthic treatment on atopy among children are conflicting (25–28) and none showed effects on wheeze or eczema, although statistical power for these outcomes was usually limited. Variable findings for atopy may be a consequence of heterogeneity between study settings in helminth species; of note, no trial has yet investigated the effects of treatment of schistosomiasis on asthma, eczema and atopy.

We designed a cluster-randomized trial, the Lake Victoria Island Intervention Study on Worms and Allergy-related Diseases (LaVIISWA; ISRCTN47196031), to investigate the impact of intensive *vs* standard anthelminthic treatment over a 3-year period on allergy-related diseases, in a setting with heavy helminth burden, in particular schistosomiasis (29). We herein report the findings of a household survey, conducted as baseline, to investigate the hypothesis that helminth infections would be inversely associated with allergy-related conditions.

Methods

Study design and procedures

LaVIISWA is being conducted in 26 fishing villages on the Lake Victoria islands of Koome subcounty, Mukono district, Uganda, a remote setting accessible in 2–3 h from Entebbe by powered canoe. Full details of the trial design are described elsewhere (29). The baseline household survey was conducted between October 2012 and July 2013, across all trial villages, immediately preceding intervention roll-out. All households in participating villages were eligible for inclusion in the survey. Available household listings were checked and updated by the research team, and simple random samples of 45 households were selected from each village. In selected households, all members were eligible for inclusion in the survey.

Questionnaires were completed regarding household features and individual social-demographic characteristics. Information regarding asthma, eczema and allergy symptoms was obtained using questions from the International Study on Allergy and Asthma in Children (ISAAC) questionnaire, with supplementary questions from the UK diagnostic criteria for atopic eczema (30, 31).

A general history and examination, including height, weight and hepatosplenomegaly, was performed. All individuals were examined for visible flexural dermatitis: for this, all team members were trained in the standardized approach described in (32). SPTs were performed on participants aged ≥1 year, using standard methods, with three allergens [*Dermatophagoides* mix, *Blomia tropicalis* and German cockroach (*Blatella germanica*)] and positive and negative controls (ALK-Abelló; supplied by Laboratory Specialities (Pty) Ltd., Randburg, South Africa). Each participant was asked for one stool sample; mid-stream urine samples were requested from all participants in the 15 villages surveyed from February 2013 onwards. Blood samples of 14 ml were obtained from individuals ≥13 years, 10 ml from children 5–12 years and 6 ml from children 1–4 years. Individuals were offered HIV counselling and testing in collaboration with local health service providers.

Ethical approval was granted by the Research and Ethics Committee of the Uganda Virus Research Institute, the Uganda National Council for Science and Technology, and the London School of Hygiene and Tropical Medicine. Individual written informed consent (for adults ≥18 years and emancipated minors, and for children by a parent or guardian) and assent (for children 8–17 years) was sought for survey participation.

Laboratory methods

Two slides from each stool sample were examined (by different technicians) using the Kato-Katz method (33). The remaining sample was suspended in ethanol and stored at −80°C to allow further investigation for *Necator americanus* and *Strongyloides*

stercoralis, and, among a subset of 200 participants, for *Ancylostoma duodenale*, using real-time polymerase chain reaction (RT-PCR) (34). Quality control for PCR assays was conducted at St Elisabeth's Hospital, Tilburg, NL. The Uganda results were comparable for *N. americanus* and *A. duodenale*, but had a lower detection rate for *S. stercoralis*. The presence of circulating cathodic antigen (CCA) of *S. mansoni* in urine was assessed (Rapid Medical Diagnostics, Pretoria, South Africa). Infection intensity based on Kato-Katz results was classified using WHO-recommended cut-offs (35). For PCR results, there are no standard cut-offs for categorizing infection intensity; however, based on results from Verweij et al. (34), individuals with $C_t > 30$ would have parasite loads difficult to detect by microscope. *Mansonella perstans* infection was determined by a modified Knott's method (36); malaria was determined by thick blood film.

IgE specific to *Dermatophagoides* and cockroach allergens was measured by ELISA (29). The lower detection limit for our in-house ELISA was 15.6 ng/ml. We used 20-fold diluted plasma samples in our assay; hence, the lower detection limit in undiluted plasma was calculated as 312 ng/ml. This was used as a cut-off to create binary variables for detectable *vs* undetectable responses for each allergen.

Statistical methods

This was a cross-sectional analysis of survey data. Outcomes were reported wheeze in the last 12 months for children <5 years and for participants ≥5 years; visible flexural dermatitis; atopy defined as positive SPT response to any allergen for participants ≥1 year; atopy assessed as concentration of asIgE and analysed both as a continuous outcome and as detectable/nondetectable using the cut-off of 312 ng/ml. Exposures for the analysis were helminth infections. The following variables were considered as potential confounders: individual socio-demographic characteristics (age, sex, birth order, number of siblings, area of birth, area resided in for first 5 years, preschool attendance; occupation, maternal tribe, paternal tribe); behavioural and clinical characteristics (hand-washing behaviour, BCG scar, maternal or paternal allergy/asthma/eczema, immunization history, breastfeeding, exposure to anthelminthic treatment *in utero*, anthelminthic treatment in last 12 months, artemisinin combination treatment for malaria in last 12 months, malaria infection, HIV infection); and household characteristics (crowding, animal ownership, asset score, indoor cooking, toilet access, drinking water source, washing water source, malaria control measures).

Assuming an average of 2.8 people per household, we expected that sampling 45 households per village would yield at least 3250 participants. For common exposures (prevalence ≥20%), assuming a design effect of 1.5 and outcome prevalence of 10%, the study would have over 80% power to detect risk ratios ≥1.5.

All analyses employed the 'svy' survey commands in Stata to allow for clustering of respondents within villages using linearized standard errors (37) and for variable village sizes using weights. Village-level weights were calculated based on the numbers of included and total households in each village. For binary outcomes, univariable and multivariable logistic regressions were used to obtain crude and adjusted odds ratios (OR) and 95% confidence intervals (CI). *P*-values were calculated using Wald tests. Adjustment was made for any potential confounder for which there was evidence of crude association with the outcome or which was considered to have a possible role, *a priori*.

Raw asIgE responses were skewed. Therefore, we used simple and multiple linear regressions to examine the association between helminth infections and $\log_{10}$ levels of asIgE, and back transformed results to obtain geometric mean ratios (GMRs) and 95% CIs. For all outcomes, the role of *S. mansoni* infection intensity was assessed using the test for trend.

The population attributable fraction (PAF) for reported wheeze due to atopy was estimated as p'(OR−1)/OR with p' the prevalence of a positive SPT response among individuals with reported wheeze and OR the odds ratio for the wheeze–SPT association. We prespecified that we would examine whether helminth infections modified the associations between the atopy and the wheeze outcomes, by fitting interaction terms in multivariable logistic regression models. We also undertook a series of additional exploratory interaction analyses between helminth infections for each outcome, in an attempt to understand the primary association findings. Finally, as most previous studies have been performed in children, we conducted an exploratory investigation into whether associations between allergy-related outcomes and between helminths and allergy-related outcomes differed by age group (<16 *vs* ≥16 years).

Results

Study participant characteristics

Of 1170 households selected, 144 (median per village 5, range 0–13) were excluded, because nobody was available to take part ($n = 74$), household members refused ($n = 34$), household was unoccupied ($n = 28$), household members' main place of residence was another selected household ($n = 6$), and household members were ill ($n = 2$). From the remaining 1026 households, 2316 individuals were surveyed. Characteristics of the participating individuals are shown in Table 1, with further details described elsewhere (29).

Reported wheeze in the last 12 months was rare (Table 1) but increased with age (Fig. 1A); 15 participants (0.7%) had visible flexural eczema (four satisfied the UK criteria for atopic eczema). Nineteen per cent of participants were atopic based on SPT with cockroach the most common allergen to elicit a response. Prevalence of positive SPT response to cockroach peaked in school-aged children while for other allergens, prevalence increased with age (Fig. 1A). Median (IQR) asIgE was 1440 (170–3990) ng/ml for *Dermatophagoides* and 220 (70–650) ng/ml for cockroach, with 73% of participants having detectable levels of *Dermatophagoides* IgE, 41% having detectable levels of cockroach IgE and 80% having detectable asIgE for either allergen.

The numbers and survey design-adjusted percentages of individuals infected with each helminth are shown in Table 1.

Table 1 Characteristics of survey participants

Characteristic	*n*/*N* (%*)
Socio-demographic characteristics	
Age in years, median (IQR)	24 (8, 32)
Male sex	1268/2316 (54.1)
Place of birth	
Fishing village	617/2302 (26.6)
Rural village	1381/2302 (59.2)
Town or city	304/2302 (14.1)
Occupation	
Child/student	737/2299 (32.8)
Housewife	125/2299 (6.0)
Fishing or lake related	836/2299 (36.6)
Shops, saloons, artisans, service providers	125/2299 (6.0)
Bars, restaurants, food providers, entertainment	152/2299 (5.8)
Agriculture, lumbering, charcoal	266/2299 (10.0)
Professional	15/2299 (0.9)
Unemployed	43/2299 (2.0)
Number of siblings, median (IQR)	5 (3, 8)
P. falciparum infection	139/2115 (7.3)
HIV infection (≥16 years)	244/1376 (17.5)
Any previous worm treatment in last 12 months	949/2284 (40.6)
Helminth infections	
S. mansoni (Kato-Katz)	1041/1996 (51.4)
S. mansoni (urine CCA)	661/917 (72.0)
S. mansoni intensity (Kato-Katz)	
Uninfected	955/1996 (48.6)
Low	429/1996 (21.0)
Moderate	288/1996 (13.7)
Heavy	324/1996 (16.6)
N. americanus (PCR)	453/1994 (21.9)
N. americanus intensity (PCR), Ct median (IQR)	35.7 (33.0–39.1)
S. stercoralis (PCR)	259/1994 (11.9)
S. stercoralis intensity (PCR), Ct median (IQR)	34.2 (31.9–7.1)
T. trichiura (Kato-Katz)	230/1996 (9.8)
T. trichiura intensity (Kato-Katz)	
Uninfected	1766/1996 (90.2)
Low	223/1996 (9.5)
Moderate	6/1996 (0.2)
Heavy	1/1996 (0.0004)
M. perstans	51/2099 (2.5)
A. lumbricoides (Kato-Katz)	27/1996 (1.2)
Allergy-related outcomes	
Wheeze in last 12 months, <5 years	12/434 (2.1)
Wheeze in last 12 months, ≥5 years	95/1862 (5.1)
Atopy (SPT)	
Any	404/1976 (19.1)
Dermatophagoides	190/1978 (9.0)
Blomia	205/1976 (9.6)
Cockroach	272.1977 (13.2)
Atopy (detectable asIgE)	
Any	1685/2116 (79.7)
Dermatophagoides	1534/2115 (72.7)
Cockroach	886/2117 (41.0)
Visible flexural dermatitis	15/2145 (0.7)

*Percentages adjusted for the survey design.

S. mansoni was most commonly detected, with infections peaking in prevalence and intensity among school-aged children (Fig. 1B), followed by *N. americanus*, *S. stercoralis*, *T. trichiura*, *M. perstans* and *A. lumbricoides* (Table 1). We did not detect any *A. duodenale* among the subgroup of 200 participants investigated. A third of those infected with *S. mansoni* based on Kato-Katz had heavy infections. For both *T. trichiura* and *A. lumbricoides,* all but seven infected individuals had light infections; therefore, we were not powered to look for associations between intensities of these helminths and the study outcomes. Infection intensities for *N. americanus* and *S. stercoralis* were generally light, with median (IQR) C_t values of 35.7 (33.0, 39.1) and 34.2 (31.9, 37.1), respectively. Based on urine CCA, 72% of 917 individuals tested were infected with *S. mansoni* (compared to 48% classified as infected by Kato-Katz in this subgroup). Of the 421 individuals who were *S. mansoni* uninfected based on Kato-Katz and for whom CCA results were available, 218 (52%) were positive on CCA and could be considered as having 'very light' infections not detected with Kato-Katz analysis of a single stool sample.

Associations between allergy-related outcomes

Key associations between allergy-related outcomes and between helminths and allergy-related outcomes are summarized in Fig. 2. Individuals with a positive SPT response to any allergen were more likely to report wheeze [OR 2.49 (95% CI: 1.43, 4.33), $P = 0.002$]; the PAF for reported wheeze associated with atopy based on SPT was 19.9%. This association was seen consistently for both under- and over-fives and for each of the three allergens used for SPT, and was stronger as the number of allergens for which participants had a positive SPT increased (*P*-value for trend test <0.001). Individuals with higher *Dermatophagoides*-specific IgE were more likely to have a positive SPT response to *Dermatophagoides* [OR for each unit increase in log *Dermatophagoides*-specific IgE 1.69 (95% CI: 1.30, 2.20), $P < 0.001$]; cockroach-specific IgE and cockroach-specific SPT response were also positively associated albeit less strongly [OR 1.19 (1.04, 1.36), $P = 0.02$]. Dermatophagoides-specific IgE level and reported wheeze (all ages) were weakly positively associated [OR 1.21 (0.96, 1.51), $P = 0.10$]; cockroach-specific IgE level and reported wheeze were inversely associated [OR 0.77 (0.64, 0.91), $P = 0.01$].

Associations between helminths and allergy-related outcomes

For reported wheeze in under-fives, we were only able to examine associations with *S. mansoni* and *T. trichiura* (as for

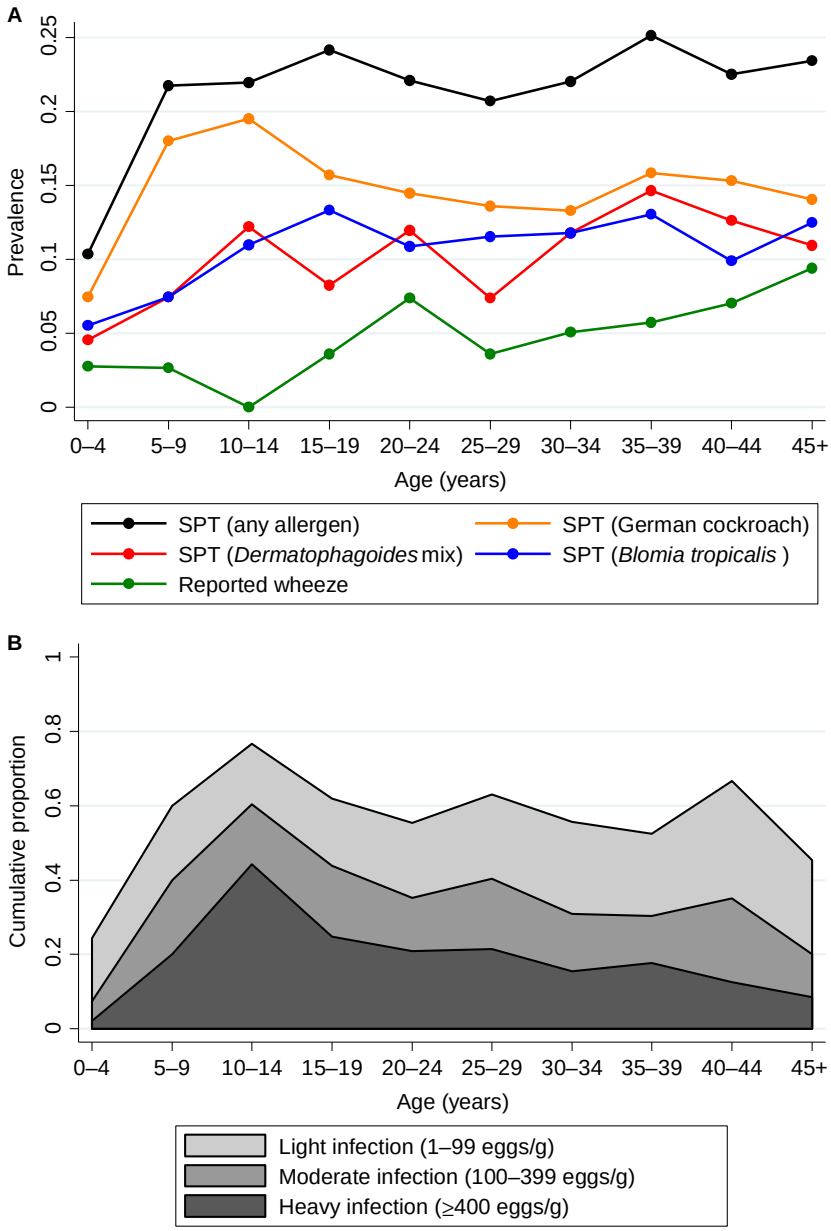

Figure 1 (A) Prevalence of positive SPT response (any allergen, German cockroach, *Dermatophagoides* mix, *Blomia tropicalis*) and reported wheeze in last 12 months, by age group. (B) Prevalence and intensity of *S. mansoni* infections, by age group.

all other helminths, no infected child had reported wheeze) and found no evidence of association with either [adjusted OR (95% CI), *P*: 2.12 (0.23, 19.20), 0.49 and 3.06 (0.65, 14.49), 0.15, respectively].

Table 2 summarizes associations between helminth infections and wheeze in over-fives, and atopy based on SPT. Table 3 summarizes associations between helminths and asIgE response [analysed as detectable *vs* nondetectable and as log (asIgE)]. *S. mansoni* was positively associated with *Dermatophagoides*-specific IgE [aOR for detectable *vs* nondetectable 1.43 (1.19, 1.72), $P < 0.001$ and aGMR from continuous analysis 1.64 (1.23, 2.18), $P = 0.001$, respectively]. There was a dose–response relationship, with individuals with the heaviest infections most likely to have high IgE (test for trend $P < 0.001$, Table 3). *T. trichiura* was positively associated with atopy based on SPT response [aOR 2.08 (1.38, 3.15), $P = 0.001$ for SPT to any allergen] with the strongest association seen for cockroach SPT. Individuals infected with *S. stercoralis* were somewhat more likely to have detectable cockroach-specific IgE [aOR 1.31 (1.00, 1.72), $P = 0.05$]. Individuals with *M. perstans* were more likely to have detectable cockroach-specific IgE and to have higher levels [aOR 2.48 (1.51, 4.07), $P = 0.001$ and aGMR 2.37 (1.39, 4.06), $P = 0.003$, respectively]. Finally, *A. lumbricoides* was posi-

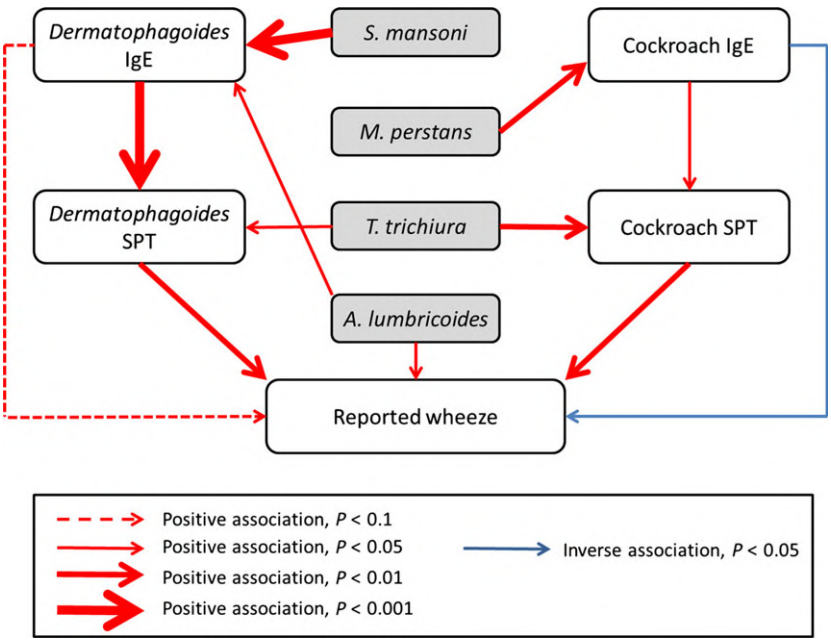

Figure 2 Summary of associations between helminths and allergy-related outcomes. Red arrows denote positive associations and blue arrows denote inverse associations, with the thickness of the arrow indicating the magnitude of the *P*-value.

tively associated with wheeze in individuals ≥5 years [aOR 6.36 (1.10, 36.63), $P = 0.04$] and with *Dermatophagoides*-specific IgE [aOR 2.58 (1.24, 5.34), $P = 0.01$ and aGMR 2.34 (1.11, 4.95), $P = 0.03$]. No inverse associations between the helminths and the allergy-related outcomes considered were seen.

Investigation of effect modification by helminths and age group

The positive association between *Dermatophagoides* IgE level and wheeze was only seen among those infected with *S. mansoni* [OR = 1.75, (1.22, 2.52), $P = 0.004$] and not in uninfected individuals [OR = 1.04 (0.81, 1.33), $P = 0.77$; interaction $P = 0.01$], Table S1. A similar pattern was seen for the association between cockroach SPT and wheeze [OR = 3.27 (2.08, 5.14), $P < 0.001$ among *S. mansoni*-infected individuals, OR = 1.37 (0.52, 3.61), $P = 0.51$ among *S. mansoni*-uninfected individuals, interaction $P = 0.09$, Table S1]. Conversely, there was some evidence that the positive association between cockroach-specific IgE and SPT was suppressed among those infected with hookworm [OR = 0.95 (0.72, 1.26), $P = 0.72$ among hookworm-infected individuals, OR = 1.38 (1.10, 1.74), $P = 0.008$ among hookworm-uninfected individuals, interaction $P = 0.11$, Table S1]. No interactive effects of other helminths were seen. The inverse association between cockroach IgE and reported wheeze was only seen among adults [OR = 0.70 (0.59, 0.83), $P < 0.001$ among those aged ≥16 years), OR = 1.40 (0.98, 2.02), $P = 0.07$ among those aged <16 years, interaction $P = 0.002$].

The positive associations between *T. trichiura* and SPT response and between *A. lumbricoides* and wheeze in over-fives were enhanced by concurrent infection with *S. stercoralis* (interaction $P = 0.004$ and $P < 0.001$, respectively; Table 4) and the former was also enhanced by concurrent hookworm (interaction $P = 0.05$; Table 4). *M. perstans* infection was associated with higher levels of *Dermatophagoides*-specific IgE in the absence of concurrent *S. mansoni* infection (interaction $P = 0.007$). There was no evidence of effect modification between age group and helminths for any outcome.

Discussion

In these remote fishing communities of Lake Victoria where helminths are highly prevalent, atopy was more common in individuals infected with *Trichuris*, schistosomiasis, *M. perstans* or *Ascaris*, and reported wheeze in the last year was more common among those with *Ascaris* infection. Our findings are in contrast to the hypothesis that chronic helminth infections protect against atopy and allergy-related diseases.

We explored various explanations for why our findings might differ from those reported by others. Concurrent infection with other helminths or pathogens could be a factor: we found some evidence that the association between *Trichuris* infection and SPT response was enhanced by concurrent infection with *S. stercoralis* or hookworm (Table 4); these results could also be interpreted as indicating that *S. stercoralis* and hookworm infections were inversely associated with atopy, in the absence of *Trichuris* co-infection. The fact that our survey was not restricted to children (unlike most other studies) is unlikely to be the explanation: although we found that the inverse association between cockroach IgE and reported wheeze was not seen in children, there was otherwise no evidence for effect modification by age. Our findings were consistent for both cockroach and dust mite allergens;

Table 2 Associations between helminth infections and (i) reported wheeze in individuals aged over 5 years, (ii) positive skin prick test to *Dermatophagoides* mix, cockroach, *Blomia tropicalis*, any allergen. Adjusted associations with $P < 0.05$ are highlighted in bold

Helminth infection status	*n*/*N* (%)*	Adjusted OR (95% CI)†·‡	*P*
Outcome: wheeze, over 5 years			
S. mansoni§			
Uninfected	39/665 (6.5)	1	
Infected	49/940 (4.7)	0.81 (0.47, 1.40)	0.44
S. mansoni intensity¶			
Uninfected	39/665 (6.5)	1	
Light	19/361 (4.6)	0.76 (0.42, 1.38)	0.64
Moderate	11/266 (4.2)	0.73 (0.32, 1.66)	(0.83)**
Heavy	19/313 (5.3)	1.00 (0.39, 2.58)	
N. americanus			
Uninfected	65/1196 (5.2)	1	
Infected	23/408 (6.3)	1.20 (0.60, 2.41)	0.60
T. trichiura			
Uninfected	78/1416 (5.4)	1	
Infected	10/189 (6.3)	1.55 (0.73, 3.30)	0.24
S. stercoralis			
Uninfected	74/1369 (5.5)	1	
Infected	14/235 (5.5)	0.81 (0.45, 1.47)	0.47
M. perstans			
Uninfected	90/1707 (5.3)	1	
Infected	4/50 (7.4)	1.27 (0.30, 5.42)	0.73
A. lumbricoides			
Uninfected	84/1585 (5.3)	**1**	
Infected	4/20 (19.2)	**6.36 (1.10, 36.63)**	**0.04**
Outcome: atopy (skin prick test positive to any allergen)			
S. mansoni§			
Uninfected	147/805 (16.5)	1	
Infected	215/965 (20.6)	1.13 (0.86, 1.47)	0.37
S. mansoni intensity¶			
Uninfected	147/805 (16.5)	1	
Light	96/395 (22.5)	1.26 (0.85, 1.86)	0.67
Moderate	56/271 (19.6)	1.02 (0.74, 1.41)	(0.96)**
Heavy	63/299 (19.2)	1.02 (0.70, 1.50)	
N. americanus			
Uninfected	290/1367 (19.5)	1	
Infected	71/400 (15.9)	0.72 (0.47, 1.10)	0.12
T. trichiura			
Uninfected	301/1567 (17.6)	**1**	
Infected	61/203 (29.7)	**2.08 (1.38, 3.15)**	**0.001**
S. stercoralis			
Uninfected	327/1532 (19.6)	1	
Infected	34/235 (12.5)	0.55 (0.29, 1.06)	0.07
M. perstans			
Uninfected	392/1900 (19.4)	1	
Infected	9/48 (16.0)	0.68 (0.28, 1.68)	0.39
A. lumbricoides			
Uninfected	356/1744 (18.7)	1	
Infected	6/26 (23.4)	1.31 (0.40, 4.26)	0.64
Outcome: atopy (skin prick test positive to *Dermatophagoides*)			
S. mansoni§			
Uninfected	68/806 (7.6)	1	
Infected	104/966 (9.9)	1.13 (0.89, 1.44)	0.31
S. mansoni intensity¶			
Uninfected	68/806 (7.6)	1	
Light	46/395 (10.0)	1.12 (0.74, 1.70)	0.74
Moderate	28/271 (10.1)	1.17 (0.76, 1.80)	(0.50)**
Heavy	30/300 (9.6)	1.11 (0.74, 1.65)	
N. americanus			
Uninfected	139/1369 (9.3)	1	
Infected	32/400 (7.3)	0.72 (0.43, 1.21)	0.20
T. trichiura			
Uninfected	147/1569 (8.4)	**1**	
Infected	25/203 (12.7)	**1.73 (1.03, 2.90)**	**0.04**
S. stercoralis			
Uninfected	152/1534 (8.9)	1	
Infected	19/235 (8.0)	0.84 (0.38, 1.86)	0.65
M. perstans			
Uninfected	184/1902 (9.1)	1	
Infected	3/48 (7.1)	0.78 (0.27, 2.26)	0.63
A. lumbricoides			
Uninfected	168/1746 (8.7)	1	
Infected	4/26 (17.6)	2.46 (0.73, 8.31)	0.14
Outcome: atopy (skin prick test positive to cockroach)			
S. mansoni§			
Uninfected	92/805 (10.5)	1	
Infected	152/966 (14.8)	1.29 (0.88, 1.90)	0.18
S. mansoni intensity¶			
Uninfected	92/805 (10.5)	1	
Light	69/395 (17.1)	1.58 (0.96, 2.61)	0.20
Moderate	41/271 (13.8)	1.14 (0.75, 1.72)	(0.95)**
Heavy	42/300 (12.8)	1.02 (0.60, 1.73)	
N. americanus			
Uninfected	19/1368 (13.1)	1	
Infected	51/400 (11.7)	0.73 (0.40, 1.31)	0.27
T. trichiura			
Uninfected	198/1568 (11.8)	**1**	
Infected	46/203 (21.8)	**1.98 (1.30, 3.01)**	**0.003**
S. stercoralis			
Uninfected	219/1533 (13.3)	1	
Infected	25/235 (9.3)	0.62 (0.32, 1.18)	0.14
M. perstans			
Uninfected	267/1901 (13.5)	1	
Infected	5/48 (9.4)	0.58 (0.21, 1.62)	0.28
A. lumbricoides			
Uninfected	240/1745 (12.8)	1	
Infected	4/26 (15.0)	1.06 (0.33, 3.37)	0.92
Outcome: atopy (skin prick test positive to *Blomia tropicalis*)			
S. mansoni§			
Uninfected	81/805 (8.5)	1	
Infected	98/965 (9.7)	1.02 (0.66, 1.59)	0.92
S. mansoni intensity¶			
Uninfected	81/805 (8.5)	1	
Light	42/395 (9.2)	0.97 (0.58, 1.63)	0.89
Moderate	23/271 (8.6)	0.95 (0.59, 1.52)	(0.65)**
Heavy	33/299 (11.2)	1.18 (0.64, 2.19)	
N. americanus			
Uninfected	139/1367 (9.2)	1	

Table 2 (continued)

Helminth infection status	*n*/*N* (%)*	Adjusted OR (95% CI)†‡	*P*
Infected	39/400 (8.8)	0.89 (0.56, 1.44)	0.63
T. trichiura			
Uninfected	155/1567 (8.8)	1	
Infected	24/203 (11.8)	1.45 (0.79, 2.56)	0.22
S. stercoralis			
Uninfected	163/1532 (9.5)	1	
Infected	15/235 (6.3)	0.59 (0.26, 1.34)	0.20
M. perstans			
Uninfected	195/1900 (9.6)	1	
Infected	7/48 (13.8)	1.37 (0.58, 3.26)	0.45
A. lumbricoides			
Uninfected	174/1744 (9.0)	1	
Infected	5/26 (21.7)	3.73 (0.91, 15.26)	0.07

*Percentages adjusted for the survey design.

†Adjusted odds ratio (OR) and 95% confidence intervals (CI) adjusted for survey design.

‡All adjusted ORs adjusted for age, sex, occupation, number of siblings. For associations with wheeze, all ORs were also adjusted for household asset score and household crowding and helminth-specific ORs were additionally adjusted as follows: *S. mansoni* – malaria infection, hand-washing behaviour, *A. lumbricoides* infection; hookworm – malaria infection, hand-washing behaviour, HIV infection, *A. lumbricoides* infection; *T. trichiura* – hand-washing behaviour; *M. perstans* – malaria infection; *A. lumbricoides* – maternal history of asthma. For associations with SPT (any and allergen-specific), all ORs were also adjusted for number of siblings, area of birth, and helminth-specific ORs were additionally adjusted as follows: *S. mansoni* – *T. trichiura* infection; hookworm – *S. mansoni* infection, *S. stercoralis* infection, *T. trichiura* infection. For associations with *Dermatophagoides* SPT, helminth-specific ORs were additionally adjusted as follows: *S. mansoni* – maternal tribe, hand-washing behaviour; hookworm – household toilet access; *T. trichiura* – maternal tribe, household toilet access; *M. perstans* – maternal tribe. For associations with cockroach SPT, helminth-specific ORs were additionally adjusted as follows: hookworm – household asset score; *T. trichiura* – household toilet access; *S. stercoralis* – household asset score. For associations with *Blomia tropicalis* SPT, ORs for *S. mansoni* and hookworm were additionally adjusted for hand-washing behaviour.

§Based on Kato-Katz.

¶Light: 1–99 eggs per gram, moderate: 100–399 eggs per gram, heavy: ≥400 eggs per gram.

***P*-value for test for trend.

thus, differences between studies in allergens used is unlikely to explain our different findings. Another possible explanation that we cannot exclude is that individuals in our study setting may have suffered more long-term and chronic infections compared to those in other studies. Finally, although hookworm infection as detected by PCR was fairly common, it was generally of low intensity; hence, this may have reduced our ability to detect associations for this helminth.

We estimate that in this setting, the population fraction of reported wheeze attributable to atopy is around 20%, lower than reported in most high-income settings, but similar to many other LICs (9, 38). Consistent with findings from other developing country settings (21), hookworm infection appeared to suppress associations between IgE and SPT for cockroach responses; however, schistosomiasis had the opposite effect, promoting associations between atopy and wheeze for both dust mite and cockroach allergens.

Helminth infections could promote atopy either by non-specifically driving the antigen presentation-T-cell-to-B-cell immune response axis towards greater production of IgE, or by inducing cross-reactive IgE. Regarding the latter, asthma severity has been shown to be related to *Ascaris* IgE levels, which correlate with mite-specific IgE (39), cross-reactivity has been demonstrated for selected *Ascaris* and mite antigens (40), and immunization of rabbits with *Ascaris* antigens induces IgE which cross-reacts with house dust mite (41). This accords with our observed association between *Ascaris* and elevated dust mite-specific IgE. Likewise, molecular modelling indicates that *S. mansoni* contains cysteine proteases homologous to the house dust mite antigen Der p 1 (42) and *S. mansoni* infection was associated with elevated house dust mite-specific IgE in our study. Cross-reactive IgE can also occur to molecules such as tropomyosin which are highly conserved between invertebrates (including nematodes, schistosomes, mites and cockroach) (43) and, consistent with cross-reactivity, the prevalence of positive SPT responses to cockroach and the heaviest *S. mansoni* infections both peaked in school-aged children in our study.

Mechanisms for a positive association between helminths and wheeze could include exacerbation of allergen-specific atopic responses or a direct response to the allergen-like helminth proteins experienced during larval migration through the lungs. The former might explain our finding that concurrent *S. mansoni* infection strengthens the association between allergen-specific IgE and wheeze. The latter may explain the association between *Ascaris* and wheeze – the long-recognized Löffler's syndrome (44) – although cross-reactivity between Ascaris and mite allergens may also contribute, as discussed above.

For each of these possible mechanisms, concurrent infection with a helminth species that down-modulates allergy-related immune responses might modify the association between pro-allergenic helminth species and allergy-related outcomes in the same way that hookworm has been observed to modify the link between allergen-specific IgE and histamine release (21). However, our interaction analyses showed little evidence of such effects: the only result consistent with this hypothesis was that *S. mansoni* infection modified the association between *M. perstans* and house dust mite-specific IgE production.

Despite the positive associations observed between helminths and allergy-related outcomes in this study, the overall prevalence of wheeze, eczema and SPT positivity was low compared with developed countries and urban settings in low or middle-income countries (8, 45). This suggests that factors other than current active helminth infection in these communities have important protective effects against allergy-related outcomes. These factors could include prenatal exposure to helminths (22, 46), exposures to a myriad of infectious, xeno-

Table 3 Associations between helminth infections and allergen-specific IgE. Adjusted associations with $P < 0.05$ are highlighted in bold

Helminth infection status	*n*/*N* (%)/geometric mean*	Adjusted OR/GMR (95% CI)†‡	*P*
Outcome: atopy (Detectable *Dermatophagoides*-specific IgE)			
S. mansoni§			
Uninfected	588/873 (67.4)	**1**	
Infected	771/1007 (76.8)	**1.43 (1.19, 1.72)**	**<0.001**
S. mansoni intensity¶			
Uninfected	588/873 (67.4)	**1**	
Light	298/411 (71.8)	**1.13 (0.89, 1.42)**	**0.002**
Moderate	216/279 (79.3)	**1.69 (1.22, 2.35)**	**(<0.001)****
Heavy	257/317 (81.0)	**1.95 (1.28, 2.95)**	
N. americanus			
Uninfected	1029/1446 (71.4)	1	
Infected	329/432 (75.9)	1.10 (0.75, 1.61)	0.60
T. trichiura			
Uninfected	1188/1659 (71.9)	1	
Infected	171/221 (76.7)	1.13 (0.82, 1.56)	0.44
S. stercoralis			
Uninfected	1169/1630 (71.5)	1	
Infected	189/248 (79.0)	1.26 (0.95, 1.67)	0.11
M. perstans			
Uninfected	1483/2044 (72.7)	1	
Infected	40/51 (83.8)	1.53 (0.68, 3.41)	0.29
A. lumbricoides			
Uninfected	1336/1853 (72.2)	**1**	
Infected	23/27 (88.0)	**2.58 (1.24, 5.34)**	**0.01**
Outcome: atopy (Detectable cockroach-specific IgE)			
S. mansoni§			
Uninfected	362/874 (41.0)	1	
Infected	425/1008 (41.6)	0.88 (0.58, 1.35)	0.56
S. mansoni intensity¶			
Uninfected	362/874 (41.0)	1	
Light	174/411 (40.4)	0.85 (0.52, 1.39)	0.92
Moderate	112/279 (43.3)	0.94 (0.48, 1.82)	(0.62)**
Heavy	139/318 (41.7)	0.89 (0.57, 1.39)	
N. americanus			
Uninfected	598/1447 (40.4)	1	
Infected	188/433 (44.2)	1.06 (0.75, 1.49)	0.73
T. trichiura			
Uninfected	690/1660 (41.1)	1	
Infected	97/222 (43.1)	1.03 (0.85, 1.24)	0.76
S. stercoralis			
Uninfected	667/1632 (40.4)	1	
Infected	119/248 (47.3)	1.31 (1.00, 1.72)	0.05
M. perstans			
Uninfected	852/2046 (40.7)	**1**	
Infected	31/51 (61.9)	**2.48 (1.51, 4.07)**	**0.001**
A. lumbricoides			
Uninfected	776/1855 (41.3)	1	
Infected	11/27 (42.6)	0.96 (0.55, 1.67)	0.87
Outcome: level of Dermatophagoides-specific IgE			
S. mansoni§			
Uninfected	253	**1**	
Infected	530	**1.64 (1.23, 2.18)**	**0.001**
S. mansoni intensity¶			
Uninfected	253	**1**	
Light	344	**1.14 (0.77, 1.69)**	**0.001**
Moderate	602	**1.94 (1.31, 2.87)**	**(<0.001)****
Heavy	817	**2.69 (1.46, 4.95)**	
N. americanus			

Table 3 (continued)

Helminth infection status	*n*/*N* (%)/geometric mean*	Adjusted OR/GMR (95% CI)†‡	*P*
Uninfected	350	1	
Infected	475	1.09 (0.65, 1.83)	0.72
T. trichiura			
Uninfected	361	1	
Infected	523	1.24 (0.78, 1.99)	0.35
S. stercoralis			
Uninfected	350	1	
Infected	613	1.27 (0.92, 1.77)	0.14
M. perstans			
Uninfected	378	1	
Infected	997	1.69 (0.73, 3.90)	0.21
A. lumbricoides			
Uninfected	370	**1**	
Infected	970	**2.34 (1.11, 4.95)**	**0.03**
Outcome: level of cockroach-specific IgE			
S. mansoni§			
Uninfected	164	1	
Infected	165	0.78 (0.54, 1.13)	0.18
S. mansoni intensity¶			
Uninfected	164	1	
Light	158	0.76 (0.51, 1.21)	0.53
Moderate	174	0.77 (0.37, 1.61)	(0.26)**
Heavy	167	0.84 (0.57, 1.22)	
N. americanus			
Uninfected	155	1	
Infected	201	1.12 (0.84, 1.48)	0.42
T. trichiura			
Uninfected	162	1	
Infected	194	1.17 (0.93, 1.47)	0.17
S. stercoralis			
Uninfected	162	1	
Infected	180	0.98 (0.62, 1.54)	0.92
M. perstans			
Uninfected	162	**1**	
Infected	428	**2.37 (1.39, 4.06)**	**0.003**
A. lumbricoides			
Uninfected	164	1	
Infected	242	1.57 (0.85, 2.89)	0.15

*For binary outcomes, figures shown are *n*/*N* with percentages adjusted for the survey design. For continuous outcomes, figures shown are geometric means adjusted for the survey design.

†For binary outcomes, figures shown are adjusted odds ratios (OR) [95% confidence intervals (CI)] adjusted for survey design. For continuous outcomes, figures shown are adjusted geometric mean ratios (GMR) (95% CI) adjusted for survey design.

‡All adjusted ORs adjusted for age, sex, occupation, area of birth. For associations with detectable *Dermatophagoides* IgE, ORs were additionally adjusted as follows: *S. mansoni* – preschool attendance, *A. lumbricoides* infection; hookworm – *S. mansoni* infection, *S. stercoralis* infection; *T. trichiura* – household crowding, animal ownership, *S. mansoni* infection, *A. lumbricoides* infection; *S. stercoralis* – household crowding, animal ownership; *A. lumbricoides* – *S. mansoni* infection. For associations with detectable cockroach IgE, ORs were additionally adjusted as follows: hookworm –*S. stercoralis* infection; *T. trichiura* – animal ownership; *S. stercoralis* – animal ownership.

All adjusted GMRs adjusted for age, sex, occupation, area of birth. For associations with *Dermatophagoides* IgE, GMRs were additionally adjusted as follows: *S. mansoni* – number of siblings, preschool attendance, hand-washing behaviour, *A. lumbricoides* infection; hookworm – number of siblings, malaria infection, *S. mansoni* infection, *S. stercoralis* infection; *T. trichiura* – household crowding, hand-washing behaviour, animal ownership, *S. mansoni* infection, *A. lumbricoides* infection; *S. stercoralis* – number of siblings, household crowding and animal ownership; *M. perstans* – malaria infection; *A. lumbricoides* – *S. mansoni* infection. For associations with cockroach IgE, GMRs were additionally adjusted as follows: *S. mansoni* – maternal history of asthma, hookworm infection; hookworm – anthelminthic treatment in last year; *T. trichiura* – HIV infection, animal ownership, hookworm infection; *S. stercoralis* – HIV infection, animal ownership, anthelminthic treatment in last year.

§Based on Kato-Katz.

¶Light: 1–99 eggs per gram, moderate: 100–399 eggs per gram, heavy: ≥400 eggs per gram.

***P*-value for test for trend.

Table 4 Interactions between helminth infections in their association with allergy-related outcomes

Helminth infection status					
S. stercoralis status	*T. trichiura* status	*n*/*N* (%)*	Adjusted OR (95% CI)†·‡	*P*	Interaction *P*-value
Outcome: SPT response for any allergen					
Uninfected	Uninfected	279/1364 (18.7)	1		0.004
	Infected	48/168 (28.0)	1.85 (1.18, 2.88)	0.01	
Infected	Uninfected	21/201 (8.9)	1		
	Infected	13/34 (40.2)	9.88 (2.27, 43.01)	0.004	
N. americanus status	*T. trichiura* status				
Uninfected	Uninfected	251/1234 (18.7)	1		0.05
	Infected	39/133 (28.4)	1.73 (1.10, 2.73)	0.02	
Infected	Uninfected	49/331 (13.0)	1		
	Infected	22/69 (32.6)	4.06 (2.09, 7.88)	<0.001	
S. stercoralis status	*A. lumbricoides* status	*n*/*N* (%)*	Adjusted OR (95% CI)†·‡	*P*	Interaction *P*-value
Outcome: wheeze, over 5 years					
Uninfected	Uninfected	73/1352 (5.5)	1		<0.001
	Infected	1/16 (7.7)	1.63 (0.13, 19.80)	0.69	
Infected	Uninfected	11/231 (4.5)	1		
	Infected	3/4 (71.6)	485.9 (5.71, 41 341.5)	0.008	
S. mansoni status	*M. perstans* status	Geometric mean (ng/ml)*	Crude GMR (95% CI)†·‡	*P*	Interaction *P*-value
Outcome: *Dermatophagoides*-specific IgE					
Uninfected	Uninfected (n = 839)	27.9	1		0.007
	Infected (n = 19)	113.7	3.12 (1.67, 5.80)	0.001	
Infected	Uninfected (n = 979)	47.6	1		
	Infected (n = 25)	57.2	0.94 (0.50, 1.78)	0.85	

*For binary outcomes, figures shown are *n*/*N* with percentages adjusted for the survey design. For continuous outcome, figures shown are geometric means adjusted for the survey design.

†For binary outcomes, figures shown are adjusted odds ratios (OR) [95% confidence intervals (CI)] allowing for survey design. For continuous outcome, figures shown are adjusted geometric mean ratios (GMR) (95% CI) allowing for survey design.

‡All adjusted OR/GMR adjusted for age, sex, number of siblings, occupation. Associations for SPT additionally adjusted for area of birth, associations for wheeze additionally adjusted for household crowding and asset score, associations for IgE additionally adjusted for area of birth, preschool attendance, malaria infection, hand-washing behaviour.

biotic or commensal organisms (47, 48), and a range of lifestyle factors (49), which await further investigation in this setting.

We found high levels of allergen-specific IgE. This could be a consequence of immunological cross-reactivity between helminth allergens and aeroallergens or a result of nonspecific stimulation of IgE production resulting from intense helminth exposure (as discussed above), or an artefact of our in-house assay. Further studies to determine the characteristics of the IgE present in this population are in progress.

Our study had some limitations. We could only evaluate helminth infections endemic to our study setting: for example, for hookworm, we could investigate associations between *N. americanus* and allergy outcomes, but not *A. duodenale*, as the latter was not found. The cross-sectional design of this survey means that, strictly speaking, we cannot tell the relative timing of allergen sensitization and helminth exposure. However, our age-prevalence profiles show that both prenatal helminth exposure and infection in infancy and early childhood are likely in this setting so prior exposure to helminths, or concurrent exposure to helminths and allergens, is likely to have occurred. Although the use of reported wheeze in the last 12 months has been validated as a proxy measure for asthma in many settings (50), there is no direct translation of the word 'wheeze' in the local language; thus, this outcome is likely to be subject to misclassification. Indeed, the increasing prevalence of reported wheeze with age that we observed could indicate that the phenotype being captured was to some extent related to chronic bronchitis rather than asthma. We investigated the use of a video questionnaire for wheeze in the study and found that agreement between the two approaches was fairly low although participants reporting wheeze had, on average, reduced levels of lung function parameters (29). Although we collected data on reported allergic rhinitis (a common disease caused by the allergens tested in our study, in some settings) in our survey and investigated it as an exploratory outcome, we found it to be uncommon and there was no evidence of association with any helminth; however, it is possible that this outcome was subject to misclassification, for similar reasons to wheeze. An additional source of

misclassification is that we used single stool samples to assess helminth infection status (51, 52). Indeed for the subgroup of participants who underwent urine CCA testing, the prevalence of *S. mansoni* was found to be much higher than when tested using Kato-Katz of the single stool sample. Also, the PCR method used had limited sensitivity for *Strongyloides*. This could have led to underestimation of the size of any true association. The study involved a large number of statistical tests, for which we made no formal adjustment; however, the consistent patterns of positive associations are unlikely to be explained by chance. Findings from our interaction analyses should be treated with caution. Although not all of these analyses were preplanned, we felt they were important to try to shed light on our unexpected findings.

In conclusion, we found that certain helminth infections were positively associated with allergy-related outcomes in this setting, with inverse associations only being seen in subgroup analyses. The LaVIISWA trial is currently ongoing and the impact of intensive *vs* standard anthelminthic treatment will be investigated in a further cross-sectional household survey in 2016. At that time, we will be able to assess not only the direct impact of worm removal on allergy-related outcomes, but also the effect of the trial interventions on the associations reported herein. If there is a causal relationship underlying the observed associations, then the allergy-related outcome prevalence might reduce with the removal of helminth infection, rather than increasing as initially hypothesized.

LaVIISWA trial team

Project leaders: Margaret Nampijja, Richard Sanya, Barbara Nerima. Statisticians and data managers: Emily Webb, Lawrence Muhangi, Beatrice Mirembe, Justin Okello, Jonathan Levin. Clinical officer: Milly Namutebi, Christopher Zziwa. Nurses: Esther Nakazibwe, Josephine Tumusiime. Internal monitor: Mirriam Akello. Field workers: Robert Kizindo, Moses Sewankambo, Denis Nsubuga. Laboratory staff: Stephen Cose, Linda Wammes, Proscovia Kabubi, Emmanuel Niwagaba, Gloria Oduru, Grace Kabami, Elson Abayo, Joyce Kabagenyi, Gyaviira Nkurunungi, Fred Muwonge, Dennison Kizito. Boatman: David Abiriga. HIV counselling and testing: Victoria Nannozi. Vector Control Programme staff: James Kaweesa, Edridah Tukahebwa. Principal investigator: Alison Elliott.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

AME conceived the study. AME, ELW, ET and M Nampijja participated in designing the parent trial. AME, M Nampijja, J Kaweesa, RK, EN and M Namutebi led and participated in the survey. GO and PK ran the field laboratory, while J Kabagenyi, GN, DK and BN participated in establishing and conducting immunological assays and PCR. JJV trained and assisted in stool PCR. MA monitored the study activities. LM managed the database. ELW conducted the statistical analysis. ELW, M Nampijja and AME drafted the manuscript and all authors reviewed and contributed to it. All authors read and approved the final version of the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Associations between allergy outcomes stratified by *S. mansoni* and *N. americanus* status.

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9.3 Supplementary information for Research paper 6 (also available in this article's online repository at https://onlinelibrary.wiley.com/doi/full/10.1111/all.12867)

9.3.1 Supplementary Table 1. Associations between allergy outcomes stratified by S. mansoni and N. americanus status.

S. mansoni				***N. americanus***			
Infection status	**OR (95% CI)**	**p**	**Interaction p-value**	**Infection status**	**OR (95% CI)**	**p**	**Interaction p-value**
Outcomes: *Dermatophagoides* SPT and reported wheeze							
Uninfected	2.18 (0.64, 7.40)	0.20	0.53	Uninfected	2.59 (1.15, 5.84)	0.02	0.71
Infected	3.32 (1.85, 5.97)	<0.001		Infected	3.37 (1.23, 9.21)	0.02	
Outcomes: *Dermatophagoides* IgE level and *Dermatophagoides* SPT							
Uninfected	1.55 (1.03, 2.35)	0.04	0.37	Uninfected	1.68 (1.21, 2.34)	0.003	0.54
Infected	1.88 (1.41, 2.51)	<0.001		Infected	1.95 (1,41, 2.69)	<0.001	
Outcomes: *Dermatophagoides* IgE level and reported wheeze							
Uninfected	1.04 (0.81, 1.33)	0.77	0.01	Uninfected	1.32 (0.99, 1.77)	0.06	0.29
Infected	1.75 (1.22, 2.52)	0.004		Infected	1.01 (0.64, 1.59)	0.96	
Outcomes: Cockroach SPT and reported wheeze							
Uninfected	1.37 (0.52, 3.61)	0.51	0.09	Uninfected	1.91 (1.05, 3.49)	0.04	0.19
Infected	3.27 (2.08, 5.14)	<0.001		Infected	3.59 (1.84, 7.02)	0.001	
Outcomes: Cockroach IgE level and cockroach SPT							
Uninfected	1.15 (0.86, 1.55)	0.34	0.47	Uninfected	1.38 (1.10, 1.74)	0.008	0.11
Infected	1.35 (1.06, 1.73)	0.02		Infected	0.95 (0.72, 1.26)	0.72	
Outcomes: Cockroach IgE level and reported wheeze							
Uninfected	0.75 (0.55, 1.02)	0.06	0.96	Uninfected	0.80 (0.60, 1.07)	0.12	0.39
Infected	0.75 (0.60, 0.95)	0.02		Infected	0.62 (0.41, 0.93)	0.02	

9.4 APPENDIX 3: Research Paper 7: The impact of intensive versus standard anthelminthic treatment on allergy-related outcomes, helminth infection intensity, and helminth-related morbidity in Lake Victoria fishing communities, Uganda: results from the LaVIlSWA cluster-randomized trial

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Student	GYAVIIRA NKURUNUNGI
Principal Supervisor	ALISON ELLIOTT
Thesis Title	HELMINTH-ALLERGY ASSOCIATIONS IN RURAL AND URBAN UGANDA: INSIGHTS FROM ANTIBODY STUDIES

If the Research Paper has previously been published please complete Section B, if not please move to Section C

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Where was the work published?	CLINICAL INFECTIOUS DISEASES		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I participated in establishing and conducting laboratory assays and in writing the manuscript (I am joint first author with Dr Sanya and Ms Hoek Spaans).

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Supervisor Signature:  Date: 15/2/2019

Clinical Infectious Diseases

MAJOR ARTICLE

The Impact of Intensive Versus Standard Anthelminthic Treatment on Allergy-related Outcomes, Helminth Infection Intensity, and Helminth-related Morbidity in Lake Victoria Fishing Communities, Uganda: Results From the LaVIISWA Cluster-randomized Trial

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Background. The prevalence of allergy-related diseases is increasing in low-income countries. Parasitic helminths, common in these settings, may be protective. We hypothesized that intensive, community-wide, anthelminthic mass drug administration (MDA) would increase allergy-related diseases, while reducing helminth-related morbidity.

Methods. In an open, cluster-randomized trial (ISRCTN47196031), we randomized 26 high-schistosomiasis-transmission fishing villages in Lake Victoria, Uganda, in a 1:1 ratio to receive community-wide intensive (quarterly single-dose praziquantel plus albendazole daily for 3 days) or standard (annual praziquantel plus 6 monthly single-dose albendazole) MDA. Primary outcomes were recent wheezing, skin prick test positivity (SPT), and allergen-specific immunoglobulin E (asIgE) after 3 years of intervention. Secondary outcomes included helminths, haemoglobin, and hepatosplenomegaly.

Results. The outcome survey comprised 3350 individuals. Intensive MDA had no effect on wheezing (risk ratio [RR] 1.11, 95% confidence interval [CI] 0.64–1.93), SPT (RR 1.10, 95% CI 0.85–1.42), or asIgE (RR 0.96, 95% CI 0.82–1.12). Intensive MDA reduced *Schistosoma mansoni* infection intensity: the prevalence from Kato Katz examinations of single stool samples from each patient was 23% versus 39% (RR 0.70, 95% CI 0.55–0.88), but the urine circulating cathodic antigen test remained positive in 85% participants in both trial arms. Hookworm prevalence was 8% versus 11% (RR 0.55, 95% CI 0.31–1.00). There were no differences in anemia or hepatospenomegaly between trial arms.

Conclusions. Despite reductions in *S. mansoni* intensity and hookworm prevalence, intensive MDA had no effect on atopy, allergy-related diseases, or helminth-related pathology. This could be due to sustained low-intensity infections; thus, a causal link between helminths and allergy outcomes cannot be discounted. Intensive community-based MDA has a limited impact in high-schistosomiasis-transmission fishing communities, in the absence of other interventions.

Clinical Trials Registration. ISRCTN47196031.

Keywords. helminths; *Schistosoma mansoni*; mass drug administration; allergy-related disease; Africa.

The prevalence of allergy-related diseases (ARD), such as eczema, rhinitis, and asthma, increased rapidly in high-income countries in the twentieth century [1] and is now increasing in tropical, low-income countries (LICs) [2]. Nevertheless, populations in LICs, particularly in rural settings, remain relatively protected [3]. Understanding this phenomenon is crucial to elucidating the causes and improving prevention of ARD.

By contrast, LICs carry the largest burden of parasitic helminth infections: these are associated with some severe and much subtle morbidity [4, 5]. Major anthelminthic mass drug administration (MDA) has taken place in the last decade but, although prevention of severe helminth-induced morbidity is important, wider benefits [6] and the sustainability of helminth control by MDA [7, 8] have been questioned.

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Certain helminth antigens are highly homologous to allergens; immunoglobulin (Ig)-E and the atopic pathway are presumed to have evolved to protect mammals against such organisms [9]. Parasitic helminths must modulate such responses to survive within mammalian hosts. Animal and human epidemiological and in vitro studies indicate that, through bystander effects of such immunomodulation, chronic helminth infection protects against atopy and ARD [10]. If helminths protect against ARD, MDA programs may adversely affect these outcomes. Observational studies, many of which indicate an inverse association between helminths and ARD, are subject to confounding and reverse causation; therefore, several groups have investigated the effects of anthelminthic treatment on ARD in clinical trials. Some studies show increased atopy after anthelminthic intervention, but 2 large, school-based, individually-randomized intervention trials focusing on soil-transmitted helminths (STH) reported no effect on atopy or ARD [11, 12]. A recent household-randomized trial of intensive albendazole for STH showed no effect on ARD, but upregulated pro-inflammatory responses and reduced immunoregulatory molecules [13].

East African fishing communities bear an intense schistosomiasis burden [14]. During a *Schistosoma mansoni* infection, adult worms reside in mesenteric blood vessels and eggs are excreted through intestinal mucosa, causing intestinal and tissue (notably liver) pathology [5]. *Schistosoma* infection has shown even stronger inverse associations with atopy than STH [15] and there is evidence of increased SPT reactivity with treatment [16], but no large-scale randomized trial on the allergy-related effects of intensively treating schistosomiasis has been conducted.

We undertook the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA; ISRCTN47196031) [17], a cluster-randomized trial of extended (3-year) intensive versus standard anthelminthic intervention, to assess the causal role of helminths in allergy-related outcomes and the benefits of intensive intervention for helminth-related morbidity in a schistosomiasis hot spot.

METHODS

Design and Setting

This was a 2-arm, open, cluster-randomized trial of intensive versus standard anthelminthic treatment conducted among fishing villages in the Koome islands, Lake Victoria, Uganda, between September 2012 and August 2016. The protocol has been published previously [17]. We randomized 26 villages 1:1 to either intensive or standard intervention. Village-level cluster-randomization aimed to minimize contamination from reinfection by untreated neighbours. Before the study, annual praziquantel treatment was offered to these communities, but hampered by logistics. In our baseline survey, 17% participants reported treatment in the prior year [17].

Interventions

Standard intervention, based on the Uganda Ministry of Health guidelines, was annual single dose praziquantel at 40 mg/kg (Cipla; CSPC OUYI Pharmaceuticals, India; AGOG Pharma, India) to community members ≥94 cm, as estimated by a height pole, plus 6 monthly single dose albendazole at 400 mg (CSPC OUYI Pharmaceuticals, India; AGOG Pharma, India; Medreich, India) to all aged ≥1 year. Intensive intervention was quarterly single dose praziquantel at 40 mg/kg to individuals ≥60 cm (to allow treatment of younger children) [18], as estimated by an extended height pole, plus quarterly triple dose albendazole (400 mg daily for 3 days) to all aged ≥1 year. Pregnant women were included in both arms, receiving single dose albendazole [19, 20].

Treatment, distributed house-to-house in collaboration with the Uganda Ministry of Health Vector Control Division, was directly observed and documented against household registers, with the exception of post–day 1 albendazole in the intensive arm.

Participants and Surveys

Leaders of all 27 Koome fishing villages gave written consent for their village's participation. Allocated interventions were given to all community members (of eligible age and height) unless they were absent, sick, or refused.

Household-based surveys were conducted at baseline [21] and after 3 years of intervention. All primary and most secondary outcomes were assessed in both baseline and outcome surveys. Smaller surveys were conducted at years 1 and 2 to assess helminth trends (Supplementary Figure). Separate random household samples were selected for each survey (overlap was possible). There was no individual participant follow-up. Surveys were conducted immediately prior to respective quarterly treatments.

Household registers were updated before each survey. Villages generally comprised an intensely-populated center and a scattered periphery. Peripheral households were excluded from surveys to avoid contamination from neighboring villages, but received allocated interventions.

Baseline survey methods (previously reported) were similar to the 3-year outcome survey described below [21]. For interim surveys, stool and blood samples were collected from community members selected using a 2-stage method: 1 person was randomly selected from each of 15 randomly-selected households per village. For the 3-year survey, 70 households per village were randomly selected using a Stata program (StataCorp, College Station). In the selected households, all members ≥1 year were invited to participate. Household heads gave permission for household participation and provided the demographic details (age, sex) of all household members. Written informed consent was obtained from all adults and emancipated minors and from parents/guardians for children, with

additional assent from children ≥8 years. For each participant, a questionnaire was completed; an examination and SPT were performed; and blood, urine, and 1 stool sample were obtained. Abdominal ultrasonography was performed on children.

Outcomes

Primary outcomes were recent (last 12 months) self-reported wheezing, stratified by age (<5 years, ≥5 years); SPT positivity to mites (*Dermatophagoides* mix, *Blomia tropicalis*) and German cockroaches (*Blattella germanica*); and allergen-specific IgE (asIgE) to *Dermatophagoides* and German cockroaches (common allergens in Uganda [22]). Secondary outcomes were visible flexural dermatitis (assessed using standardized procedures); helminth infections; haemoglobin; growth (height-for-age, <20 years; weight-for-age, <11 years; and weight-for-height, <6 years, z-scores); and hepatosplenomegaly (by palpation). An additional secondary outcome, schistosomiasis-related liver and spleen morbidity assessed by abdominal ultrasonography (<18 years), was included after trial interventions commenced, when additional funding became available. Exploratory outcomes were recent urticaria and rhinitis. For logistical reasons, we could not provide infant vaccines ourselves or obtain post-immunization samples at consistent timepoints, so planned vaccine response secondary outcomes are not reported. Details on outcome ascertainment are provided (Supplementary Methods).

Randomization

At a public ceremony, 1 village was randomly selected for piloting while 26 were randomized 1:1, using restricted randomization to balance village size, prior praziquantel treatment, and distance from the sub-county health center [17] (Supplementary Methods).

Statistical Methods

For the outcome survey, we planned to sample 1540 individuals per arm (Supplementary Methods). Data were analyzed using Stata v14.0. Baseline characteristics were tabulated. Characteristics of survey participants were compared with those of non-participants by chi-squared tests. Treatment uptake was calculated both by village and treatment round as the number of people receiving treatment divided by the total number of residents.

Trial analyses were done at the cluster level. Crude and adjusted analyses (adjusting for sex, age, and the corresponding baseline summary measure of the outcome, where available) were performed. For binary outcomes, risk ratios (RRs) were calculated as the mean of the intensive-arm cluster proportions divided by the mean in the standard arm, with 95% confidence intervals (CI) calculated using a Taylor series approximation for the standard error and *P* values from unpaired *t*-tests. Where the distribution of cluster proportions was skewed, the log-cluster proportions were compared and the results were back-transformed. A 2-stage approach was used for adjusted analyses [23] (Supplementary Methods).

For continuous outcomes, intervention effects were quantified as the differences in mean outcome between trial arms, with 95% CIs calculated using the *t*-distribution. Non–normally distributed continuous outcomes were log-transformed and the results were back-transformed to obtain geometric mean ratios. For ordered categorical outcomes, a proportional-odds model was used.

Trial analyses were conducted in 2 populations: the primary analysis population (intention-to-treat population) included all individuals. The secondary analysis population comprised all individuals who had lived in their village throughout (or were born into their village during) the intervention period (per-protocol analysis).

Using a cluster-level approach [24], we conducted post hoc subgroup analyses by age group (<4 years, ≥4 years) for the primary outcomes to assess whether intervention effects differed among those exposed to differential anthelminthic interventions from birth.

Ethics Statement

Ethical approval was given by the Uganda Virus Research Institute (GC127), Uganda National Council for Science and Technology (HS 1183), and London School of Hygiene and Tropical Medicine (6187).

RESULTS

Participants and Intervention Uptake

Characteristics assessed in the baseline survey (October 2012–July 2013) were balanced between trial arms, with the exception that, compared to villages in the intensive arm (intensive villages), villages in the standard arm (standard villages) had fewer public toilets but contained more households with private toilets [17].

Figure 1 summarizes the treatment uptake. Both the praziquantel and albendazole uptake increased during the trial. The mean uptake per round was 63% for praziquantel and 64% for albendazole (intensive villages), compared to 56% and 73% (standard villages). In standard villages, the albendazole uptake was lower in the treatment rounds where praziquantel treatment was also given. Reported receipt of ≥1 dose of praziquantel in the preceding year was higher in intensive, compared to standard, villages (93% versus 75%, respectively). Reported receipt of ≥1 dose of albendazole was universally high (99% versus 98%, respectively).

Between September 2015 and August 2016, 70 households from each village were randomly selected for the outcome survey (Figure 2): 84 (5%) households refused to participate, 17 (1%) consented but no demographic data were captured, and 300 (17%) had no members that could be contacted. The remaining 1419 participating households contained 3566 residents aged ≥1 year. Overall, 3350 (94%) household members provided data for at least 1 primary outcome (recent wheezing

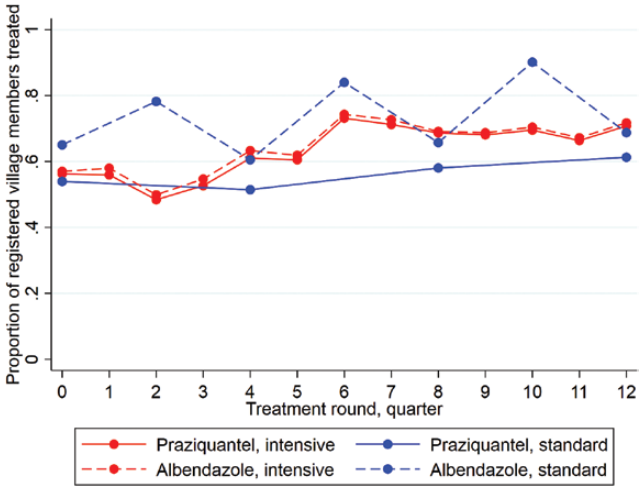

Figure 1. Praziquantel and albendazole treatment coverage, by trial arm and treatment round.

3323 [99%], SPT 3037 [91%], IgE 2955 [88%]), with numbers balanced between trial arms (Figure 2). Further details of participant characteristics are provided (Supplementary Material).

Outcome survey participant characteristics were comparable between trial arms (Table 1). Only 8 villages had access to any non-lake water supply, with public toilets available in 11 villages and private toilet access limited. The participant median age was 24 years (interquartile range 8–34) and 52% were male. Most participants (71%) had lived in their village throughout the trial. Migration between trial arms was low (1.5%). The adult human immunodeficiency virus prevalence was 22% and the reported maternal history of allergy, eczema, or asthma was 16%.

Impact of Intensive Versus Standard Anthelminthic Treatment on Primary Outcomes

The prevalence of reported wheezing among ≥5-year-olds was 3%, with little difference between trial arms (Table 2). There were 9 individuals <5 years old who reported wheezing; no formal analysis was done for this outcome. Regarding atopy, 19% participants had a positive SPT to ≥1 allergen. Of those tested using ImmunoCAP, 54% were positive (IgE > 0.35kUa/L) for either cockroach or dust mite allergens. Enzyme-linked immunosorbent assay (ELISA) and ImmunoCAP results were positively correlated for both dust mites and cockroaches (Spearman's correlation coefficient 0.32 and 0.29, respectively). There was no effect of intensive versus standard treatment on atopy (by SPT or IgE; Table 2). For all primary outcomes, there remained little evidence of a difference between trial arms in the per-protocol analysis (Supplementary Table 1) or among age groups (Supplementary Table 2), although RRs for SPT responses to individual allergens increased in both the per-protocol analysis and in children <4 years old.

Impact of Intensive Versus Standard Anthelminthic Treatment on Secondary and Exploratory Outcomes

The *Schistosoma mansoni* infection prevalence was lower in the intensive-treatment villages when assessed by stool Kato

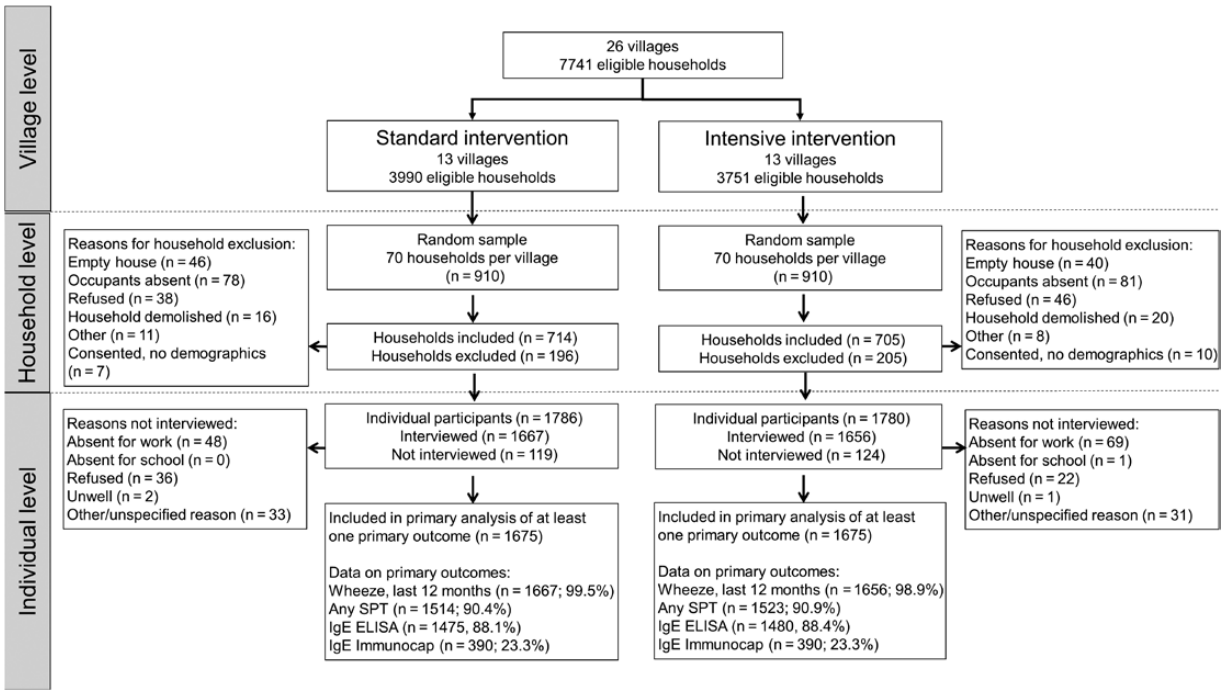

Figure 2. Trial flowchart. Abbreviations: ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; SPT, skin prick test positivity.

Table 1. Characteristics of Outcome Survey Participants

	Standard Arm		Intensive Arm	
Cluster-level Characteristics	(n = 13)		(n = 13)	
Mean no. of households per village (range)	307	(124–882)	289	(87–544)
Mean no. of participating households (range)	55	(48–63)	54	(48–64)
Mean no. of individuals resident in participating households (range)	137	(89–161)	137	(85–177)
Mean no. of individuals included in analysis (range)	129	(84–150)	129	(79–169)
Villages with any public toilets	5	38%	6	46%
Median no. of public toilets (range)	0	(0–16)	0	(0–20)
Median no. of private toilets (range)	8	(0–59)	3	(1–29)
Water supply other than lake	3	23%	5	38%
Piped water	2	67%	2	40%
River or open spring	1	33%	2	40%
Open well	0	0%	1	20%
Household-level characteristics	(n = 714)		(n = 705)	
Median no. of household members (IQR)	2	(1–3)	2	(1–3)
Individual-level characteristics	(n = 1675)		(n = 1675)	
Sex, male	881	53%	857	51%
Age in years, grouped				
0–4	283	17%	264	16%
5–9	173	10%	219	13%
10–14	66	4%	115	7%
15–19	102	6%	79	5%
20–24	212	13%	179	11%
25–29	239	14%	216	13%
30–34	198	12%	211	13%
35–39	175	10%	140	8%
40–44	86	5%	106	6%
45+	141	8%	146	9%
Place of birth (mv 9, 19)				
This fishing village	439	26%	477	29%
Other fishing village	48	3%	20	1%
Other rural village	1021	61%	1002	61%
Town	127	8%	127	8%
City	31	2%	30	2%
Has remained in village during intervention period (mv 9, 19)	1190	71%	1170	71%
Has lived in other trial arm during intervention period (mv 9, 19)	18	1%	32	2%
Maternal history of allergic diseases (mv 9, 19)				
No history	1193	72%	1204	73%
History of asthma, eczema or allergies	258	15%	266	16%
Don't know	215	13%	186	11%
Paternal history of allergic diseases (mv 9, 19)				
No history	1248	75%	1244	75%
History of asthma, eczema or allergies	145	9%	155	9%
Don't know	273	16%	257	16%
Occupation, grouped by type (mv 8, 19)				
Child, not at school	289	17%	275	17%
Student	257	15%	345	21%
Housewife	120	7%	101	6%
Fishing or lake related	564	34%	467	28%
Shops, saloons, artisans, service providers	118	7%	102	6%
Bars, restaurants, food providers, entertainment	114	7%	103	6%
Agricultural, lumbering, charcoal	157	9%	201	12%
Professional	11	1%	19	1%
Unemployed	37	2%	43	3%
Treated with albendazole in the last 12 months (mv 360, 253)	1291	98%	1404	99%
Treated with praziquantel in the last 12 months (mv 355, 253)	989	75%	1318	93%
Malaria treatment with coartem (mv 190, 167)	708	42%	747	45%
Malaria positivity by blood smear (*Plasmodium falciparum*; mv 213, 214)	50	3%	52	4%

Table 1. Continued

Cluster-level Characteristics	Standard Arm (n = 13)		Intensive Arm (n = 13)	
Individuals aged 13 years and over	(n = 1176)		(n = 1112)	
Frequency of lake contact (mv 9, 19)				
Every day	911	78%	776	71%
Almost every day	126	11%	147	13%
Once a week	95	8%	124	11%
Once a month	30	3%	35	3%
Less than once a month	4	0%	10	1%
Never	1	0%	1	0%
Individuals aged 18 years and over	(n = 1116)		(n = 1041)	
HIV+ (mv 173, 176)	192	20%	198	23%
HIV+ on ART	90	47%	103	52%
HIV+ not on ART	93	48%	90	45%
HIV+ not known if receiving ART	9	5%	5	3%

Numbers for mvs are in the standard and intensive arms, respectively.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; mv, missing values; no., number.

Katz (23% versus 39%, respectively; adjusted RR 0.70, 95% CI 0.55–0.88; Table 3) and stool polymerase chain reactions (39% versus 60%, respectively; adjusted RR 0.76, 95% CI 0.65–0.88), but urine circulating cathodic antigen positivity remained high and similar across trial arms (both 85%; Table 3), indicating that the intensive treatment was more effective than the standard treatment in reducing heavy-intensity *Schistosoma* infections, particularly in younger age groups, but had little impact on the light-infection prevalence (Figure 3). The incidence of *Schistosoma* infections was lower in both trial arms, compared to baseline, with 49% and 23% pre- and post-intervention in the intensive arm, respectively, and 56% and 39% in the standard arm, respectively. Our interim survey data suggested a greater initial reduction in intensive villages, which then plateaued, and

Table 2. Impact of Intensive Versus Standard Anthelminthic Treatment on Primary Outcomes

Outcome	n/N (%)/Geometric Mean		Unadjusted		Adjusted for Outcome at Baseline by Age and Sex[a]	
	Standard	Intensive	RR/GMR (95% CI)	*P* Value	RR/GMR (95% CI)	*P* Value
Wheeze (age ≥5 years)[b]	44/1384 (3.2%)	43/1392 (3.1%)	1.06 (0.61–1.87)	.82	1.11 (0.64–1.93)	.69
Wheeze (age <5 years)	6/284 (2.1%)	3/264 (1.1%)				
Atopy (SPT)						
SPT positivity to any allergen	273/1514 (18.0%)	303/1523 (19.9%)	1.09 (0.83–1.44)	.51	1.10 (0.85–1.42)	.46
SPT positivity to *Dermatophagoides*	162/1514 (10.7%)	164/1523 (10.8%)	0.98 (0.72–1.35)	.92	1.00 (0.74–1.36)	.99
SPT positivity to *Blomia tropicalis*	102/1514 (6.7%)	127/1522 (8.3%)	1.26 (0.83–1.90)	.26	1.27 (0.85–1.91)	.22
SPT positivity to German cockroach	156/1513 (10.3%)	194/1522 (12.8%)	1.24 (0.87–1.77)	.20	1.22 (0.87–1.71)	.21
Atopy (IgE detected by ImmunoCAP)						
Dermatophagoides or cockroach positivity (>0.35 kUa/L)	214/390 (54.9%)	210/390 (53.9%)	0.97 (0.83–1.13)	.67	0.96 (0.82–1.12)	.60
Dermatophagoides positivity (asIgE > 0.35 kUa/L)	134/390 (34.4%)	130/390 (33.3%)	0.95 (0.76–1.20)	.67	0.96 (0.77–1.19)	.68
German cockroach positivity (asIgE > 0.35 kUa/L)	201/390 (51.5%)	192/390 (49.2%)	0.94 (0.80–1.11)	.47	0.94 (0.79–1.11)	.42
Concentration of asIgE to *Dermatophagoides* (kUa/L)[c]	GM: 0.158	GM: 0.129	0.78 (0.51–1.17)	.22	0.77 (0.52–1.13)	.17
Concentration of asIgE to German cockroach (kUa/L)[c]	GM: 0.342	GM: 0.289	0.82 (0.55–1.22)	.31	0.81 (0.55–1.20)	.28
Atopy (IgE detected by in house ELISA)						
Concentration of asIgE to *Dermatophagoides*[d]	GM: 60.3	GM: 73.8	1.13 (0.36–3.50)	.83	1.17 (0.39–3.51)	.78
Concentration of asIgE to German cockroach[d]	GM: 72.4	GM: 161.0	1.98 (0.59–6.63)	.25	1.51 (0.45–5.04)	.49

Abbreviations: asIgE, allergen-specific IgE; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GM, geometric mean; GMR, geometric mean ratio; IgE, immunoglobulin E; RR, risk ratio; SPT, skin prick test positivity.

[a]Atopy outcomes assessed by IgE were adjusted for age and sex only.

[b]For this outcome, a natural log transformation was applied to village-level proportions to correct skewed distributions, and the data in parentheses are the geometric means of village proportions.

[c]Log10(+0.001) transformation at the individual level.

[d]Log10(+1) transformation at the individual level.

Table 3. Impact of Intensive Versus Standard Anthelminthic Treatment on Helminths, Clinical Outcomes, Hepatosplenomegaly by Palpation, and Anthropometry

Outcome	n/N (%)/Arithmetic Mean		Unadjusted		Adjusted for Outcome at Baseline by Age and Sex	
	Standard	Intensive	RR/Mean Difference (95% CI)	*P* Value	RR/Mean Difference (95% CI)	*P* Value
Helminth infections						
Schistosoma mansoni, stool Kato Katz	523/1355 (38.6%)	323/1396 (23.1%)	0.64 (0.43–0.94)	.02	0.70 (0.55–0.88)	.003
Schistosoma mansoni, stool PCR	797/1353 (59.9%)	541/1394 (38.8%)	0.68 (0.52–0.89)	.007	0.76 (0.65–0.88)	.001
Schistosoma mansoni, urine CCA	1229/1444 (85.1%)	1216/1435 (84.7%)	0.99 (0.91–1.08)	.85	1.00 (0.93–1.08)	.93
Hookworm, stool PCR[a]	147/1353 (10.9%)	112/1394 (8.0%)	0.54 (0.28–1.02)	.06	0.55 (0.31–1.00)	.05
Strongyloides stercoralis, stool PCR	112/1353 (8.3%)	78/1394 (5.6%)	0.74 (0.50–1.11)	.14	0.78 (0.54–1.14)	.21
Trichuris trichiura, stool Kato Katza	137/1355 (10.1%)	108/1396 (7.7%)	0.91 (0.40–2.09)	.82	0.85 (0.48–1.50)	.55
Ascaris lumbricoides, stool Kato Katz	11/1355 (0.8%)	3/1396 (0.2%)				
Clinical outcomes						
Visible flexural dermatitis	1/1558 (0.1%)	4/1553 (0.3%)				
Haemoglobin	14.0	13.9	-0.06 (-0.37–0.25)	.70	0.00 (-0.24–0.25)	.97
Anthropometry						
Height-for-age z-score, age 1–19 years	-0.48	-0.49	-0.01 (-0.20–0.19)	.95	0.02 (-0.16–0.20)	.83
Weight-for-age z-score, age 1–10 years	-0.06	-0.17	-0.11 (-0.31–0.09)	.27	-0.05 (-0.23–0.12)	.52
Weight-for-height z-score, age 1–5 years	0.15	0.19	-0.09 (-0.43–0.26)	.62	-0.06 (-0.40–0.28)	.72
Hepatosplenomegaly, palpation						
Hepatomegaly, palpation	100/1546 (6.5%)	98/1546 (6.3%)	0.97 (0.71–1.32)	.83	0.96 (0.70–1.32)	.80
Splenomegaly, palpation	87/1549 (5.6%)	63/1547 (4.1%)	0.73 (0.43–1.25)	.20	0.70 (0.43–1.15)	.13
Hepatosplenomegaly, palpation[a]	22/1548 (1.4%)	14/1548 (0.9%)	0.85 (0.52–1.39)	.49	0.78 (0.47–1.30)	.33
Reported clinical outcomes (exploratory)						
Urticaria, last 12 months	162/1667 (9.7%)	172/1656 (10.4%)	1.06 (0.86–1.30)	.59	1.06 (0.88–1.27)	.51
Rhinitis, last 12 months	78/1667 (4.7%)	74/1656 (4.5%)	1.02 (0.73–1.42)	.92	1.00 (0.74–1.36)	.99

Abbreviations: CCA, circulating cathodic antigen; CI, confidence interval; PCR, polymerase chain reaction; RR, risk ratio.

[a]For this outcome, a natural log transformation was applied to village-level proportions to correct skewed distributions.

a gradual reduction in standard villages (Figure 3). The STH prevalence was relatively low. The intensive treatment reduced hookworm prevalence; no significant reductions were seen for other nematodes (among all participants; Table 3). There was no impact of the intensive versus standard treatment on anthropometric or clinical outcomes, including hepatosplenomegaly,

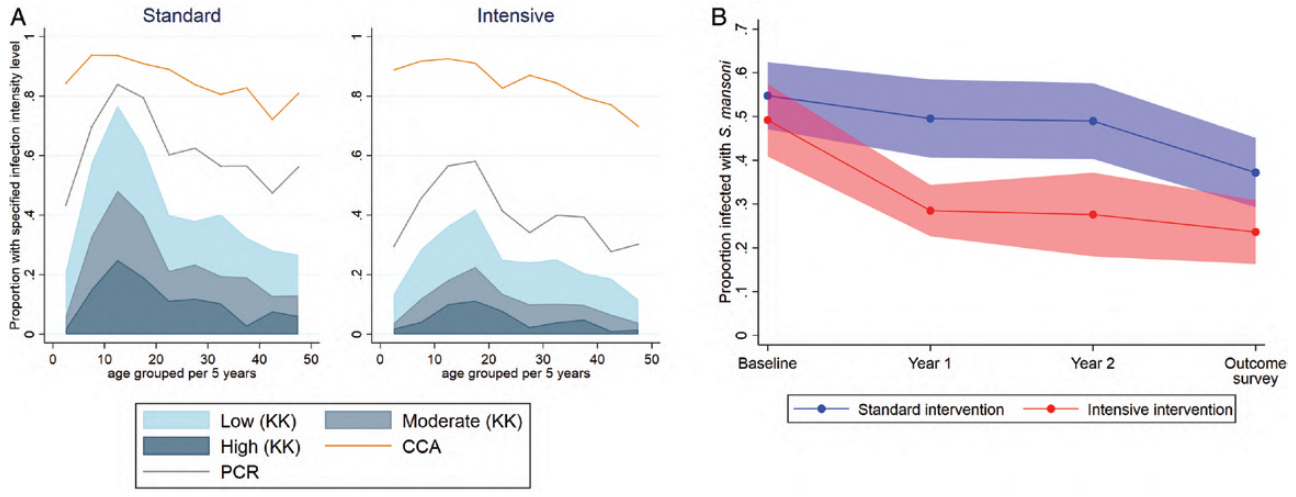

Figure 3. (*A*) Intensity of schistosomiasis infection in the outcome survey, by age group and trial arm, with prevalence assessed by KK examination of a single stool sample, PCR, and urine CCA. (*B*) Prevalence of *Schistosoma mansoni* infection over time (pre-intervention baseline survey, interim survey at 1 year, interim survey at 2 years, outcome survey at 3 years), by trial arm. Data are shown as the mean of village prevalences over time +/- 95% confidence intervals, assessed using KK analysis of a single stool sample (with duplicate slides) at each time point. Abbreviations: CCA, circulating cathodic antigen; KK, Kato Katz; PCR, polymerase chain reaction.

assessed either by palpation (Table 3) or ultrasound (among children; Supplementary Table 3). The per-protocol analysis did not yield any hitherto-unseen differences (Supplementary Table 4).

Serious Adverse Events

In total, 77 739 praziquantel treatments and 102 219 albendazole treatments were given. There were 4 serious adverse events reported (2 in each trial arm), all among adults and within 2 days of treatment: gastrointestinal symptoms leading to hospitalization (1) or requiring intravenous fluids (1); abdominal pain and vaginal bleeding in a non-pregnant woman (1); and vaginal bleeding 1 day after treatment in a pregnant woman, followed by delivery 3 days later (probably premature) and subsequent neonatal death (1). Clinic records suggested that this last woman had concurrent malaria, but this remained unconfirmed.

DISCUSSION

We report the first trial to address the community-level effects of intensive anthelminthic MDA in a high–*Schistosoma mansoni*–transmission setting. After 3 years, we found no effect of an intensive, compared to standard, intervention on allergy-related or helminth-associated disease outcomes. Intensive, compared to standard, treatment with praziquantel achieved a substantial reduction in *S. mansoni* intensity, most marked after 1 year, but infection remained almost universal. Intensive, compared to standard, treatment with albendazole achieved a modest reduction in hookworm prevalence, but had little impact on *Trichuris* or *Strongyloides*.

The prevalence of wheezing was lower than anticipated based on previous reports [25], limiting power for this outcome. However, the understanding of "wheeze" in our study communities was poor: there are no words for wheeze or asthma in the vernacular and asthma is rare. That said, there was no effect of the intensive intervention on wheezing, and no increase in wheezing during the intervention (5% at baseline [13], 3% after 3 years). These results provide reassurance that anthelminthic MDA is unlikely to have an immediate adverse effect on asthma among high-schistosomiasis-transmission communities, although no conclusions can be drawn on the impact of effective, universal *S. mansoni* removal.

SPT positivity was common. There was no increase in SPT positivity during the intervention (19% at baseline [13], 18% and 20% in the standard and intensive arms, respectively, after 3 years). There was a suggestion, especially in the per-protocol analysis and in under–4 year olds, that SPT responses increased with intensive treatment. This could be a chance finding, since a substantial number of (planned) statistical tests were conducted. This warrants more detailed investigation, as it may presage the emergence of increased atopy and ARD when helminth infections are more completely cleared. The effect of treatment may have differed based on pre-treatment infection intensity [26]. We could not assess this hypothesis, because our study was not a cohort of individual subjects.

Despite our emphasis on schistosomiasis and on long-term, community-based intervention, our results accord with previous, shorter-term trials focussing on STH [10]. However, it seems premature to conclude that high helminth prevalence has no causal link with the low ARD prevalence in LICs, given the strong effects and demonstrated mechanisms in animal models and experiments using human samples in vitro [27].

The most obvious explanation for a lack of impact on allergy-related (or helminth-associated) diseases is a failure to clear helminth infections. All villages were continuously exposed to *S. mansoni*–infested lake water because of a lack of alternative safe water, an involvement in fishing, and open defecation due to a scarcity of latrines. Although single- and first-dose treatments were directly observed, compliance was imperfect; albendazole uptake in the standard arm was lower in the rounds where praziquantel was given, indicating that villagers were averse to the praziquantel side effects. Furthermore, we cannot rule out the possible role of reduced drug efficacy [28, 29]. However, as a differential effect on helminth intensity was achieved, particularly for schistosomiasis, our results cast doubt on the extent to which intensity reduction (without elimination) substantially modifies the overall immunological or pathological effects in high-schistosomiasis-transmission settings.

Other factors contributing to the lack of impact on allergy-related outcomes may include the long-term immunological effects of helminth exposure through a persistence of antigen or through epigenetic changes in immunological pathways [30]. Also, in tropical, low-income settings, numerous other exposures—including immunomodulating infections such as malaria, exposure to dirt and domestic animals, or the microbiome profile—may impact allergy-related outcomes, such that modifying helminth exposure alone may have a limited impact [31].

A recent meta-analysis examined the effects of schistosomiasis treatments on related morbidity [32]. The results indicated wide-ranging benefits, with an increased impact when egg reduction rates were greatest and, for anemia and chronic morbidities, when treatments were repeated over periods of greater than 24 months. Thus, we were disappointed that, despite a differential reduction in schistosome intensity, we found no evidence that 3 years of intensive (compared to standard) intervention achieved improvement in any morbidity measure. This adds to the evidence base showing the limited effects of MDA on such outcomes at the community-level. We identified surprisingly little severe *Schistosoma*-related morbidity in this community, despite intense infections, consistent with earlier work from Lake Victoria communities; it is possible that intensive interventions would have a greater benefit in settings (such as Lake Albert) where severe pathology is more common [33].

Our experience emphasizes that MDA may struggle to eliminate helminths as a public health problem, especially in high-transmission environments. The substantial decline in *S. mansoni* infections (as measured by Kato Katz) achieved in year 1 led us to hope that intensive intervention could make an important contribution to schistosomiasis control in these challenging hot spots. The subsequent plateau and persistent infection (as measured by by circulating cathodic antigen) were disheartening. This phenomenon (a large drop in prevalence, followed by a subsequent plateau) has also been reported in Kenyan districts bordering Lake Victoria [34]. Besides reinfection, the possibility of selection for praziquantel-resistant or -tolerant strains is of concern [35]. A radically different approach is needed, with complementary interventions, including improved water supplies and sanitation, behavior changes, and vector controls, as well as an effective vaccine against schistosomiasis [36].

Observational analyses addressing the effects of helminths remain limited by confounding by poverty and environment. Our strategy aimed to pinpoint helminth effects by randomizing their treatment, but was constrained by difficulties in achieving removal. Trials designed so that helminths are cleared in settings where reinfection can be avoided, and with substantial follow-up, are needed for a full understanding of the risks and benefits of deworming.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

The LaVIISWA trial team project leaders, physicians, and post-doctoral scientists are Richard Sanya, Margaret Nampijja, Harriet Mpairwe, Geraldine O'Hara, and Barbara Nerima; the statisticians and data managers are Emily Webb, Remy Hoek Spaans, Lawrence Muhangi, Lawrence Lubyayi, Helen Akurut, Fatuma Nalukenge, Beatrice Mirembe, Justin Okello, Sebastian Owilla, Jonathan Levin, and Stephen Nash; the clinical officers are Milly Namutebi and Christopher Zziwa; the nurses are Esther Nakazibwe, Josephine Tumusiime, Caroline Ninsiima, Susan Amongi, Grace Kamukama, Susan Iwala, and Florence Akello; the internal monitor is Mirriam Akello; the field workers are Robert Kizindo, Moses Sewankambo, and Denis Nsubuga; the laboratory staff and collaborators are Stephen Cose, Linda Wammes, Prossy Kabuubi Nakawungu, Emmanuel Niwagaba, Gloria Oduru, Grace Kabami, Elson Abayo, Eric Ssebagala, Fred Muwonge Kakooza, Joyce Kabagenyi, Gyaviira Nkurunungi, Angela Nalwoga, Dennison Kizito, John Vianney Tushabe, Jacent Nassuuna, Jaco Verweij, Serge Versteeg, and Ronald van Ree; the social scientist is Edward Tumwesige; the ultrasonographer is Simon Mpooya; the boatman is David Abiriga; the driver is Richard Walusimbi; human immunodeficiency virus counseling and testing is provided by Victoria Nannozi and Cynthia Kabonesa; the Vector Control Programme staff are James Kaweesa and Edridah Tukahebwa; the administrative management is done by Moses Kizza; and the principal investigator is Alison Elliott.

A. M. E. conceived the study. A. M. E., E. L. W., E. T., R. E. S., M. N., and H. M. participated in designing the trial. R. E. S., A. M. E., M. N., G. O'Hara, C. Z., R. K., J. Kaweesa, E. Nakazibwe, J. T., and F. M. K. led and participated in the surveys. G. Oduru, P. K. N., E. A., and E. Niwagaba ran the field laboratory, while G. N. and J. Kabagenyi participated in establishing and conducting immunological assays and polymerase chain reactions. J. V. trained and assisted in stool polymerase chain reactions. R. vR. contributed to the testing of allergen-specific responses. M. A. monitored the study. L. L. managed the database. E. L. W., S. N., and R. H. S. conducted the statistical analysis. R. E. S., G. N., R. H. S., E. L. W., and A. M. E. drafted the manuscript, and all authors reviewed and contributed to it. All authors read and approved the final version of the manuscript.

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9.5 Supplementary information for Research paper 7 (also available in this article's online repository at https://doi.org/10.1093/cid/ciy761)

9.5.1 Supplementary Methods

9.5.1.1 Outcome assessment

Recent wheeze was assessed by ISAAC (International Study of Asthma and Allergies in Childhood)[1] questionnaire. Visible flexural dermatitis was assessed by using interview questions adopted from the UK diagnostic criteria on atopic eczema and by direct physical examination. Staff were trained in diagnosis of visible flexural dermatitis using the on-line tool by Williams.[2] Pictures of lesions were taken for a second opinion. SPT reactivity to allergens (ALK-Abelló, supplied by Laboratory Specialities (Pty) Ltd, South Africa) was assessed using standard methods. SPT outcomes were examined as any positive response versus none and as positive versus negative for individual allergens. Ultrasonography was performed using a portable machine (*Aloka SSD-900*, 3.5 mH curvilinear probe, Hitachi Aloka Medical, Japan) using the Niamey protocol.[3]

Total IgE and asIgE (*D. pteronyssinus*- and *B. germanica*-specific) were measured by ImmunoCAP® (Phadia AB, Sweden) in a random sample of 780 participants (30 per village; selected from those with SPT data and adequate sample volume) and all participants by in-house ELISA.[4] ImmunoCAP outcomes were examined as any positive response versus none (using the standard cutoff of 0.35kUa/L), as positive versus negative for individual allergens, and as continuous variables.

Intestinal helminth infection was investigated by the Kato-Katz method (two slides per sample, read by different technicians)[5] and by stool PCR for *S. mansoni, Strongyloides stercoralis* and *Necator americanus.*[6] Urine was assessed for circulating cathodic antigen (CCA, Rapid Medical Diagnostics, South Africa) of *S. mansoni*. *S. haematobium* is not present in the study area.[7] *Mansonella perstans* was determined by the modified Knott's method,[8] malaria by thick film. Voluntary HIV counselling and testing was offered

to all participants. Haemoglobin was determined by HemoCue® (HemoCue AB, Angelholm, Sweden).

9.5.1.2 Randomisation: further details

A computer-based system was used to generate all possible allocations for assigning 13 villages to each arm of the trial, satisfying restriction criteria. A list of 1000 of these possible allocations, selected at random from the full list, was provided for the randomisation ceremony. One of these was selected by village leaders using numbered balls drawn from an opaque bag (ten balls, numbered 0 to 9, selected separately for each digit).

9.5.1.3 Sample size justification

For the final survey, we planned to sample 1540 individuals per arm. Based on baseline survey data,[9] estimates for primary outcome prevalence in the standard arm were recent wheeze over the age of five years 5%, SPT positivity 20% and detectable asIgE 40%. Assuming a conservative coefficient of variation (k) of 0.3, a sample size of 1540 individuals per arm would give over 80% power to detect (at 5% significance level) a 1.5-fold difference for SPT positivity and detectable asIgE and a 1.8-fold difference for wheeze. From the baseline survey, we calculated that randomly selecting 70 households per village would yield the planned sample size (allowing for absenteeism).

9.5.1.4 Approach for adjusted analysis of trial outcomes

Adjusted analysis was done using a two-stage approach as described by Hayes and Moulton. For binary outcomes, in the first stage logistic regression of individual-level outcomes was done, including covariates to be adjusted for (but not trial arm allocation) and from this risk ratio-residuals (the ratio between observed and predicted prevalence for each cluster) were calculated. In the second stage, these risk ratio-residuals were used as the data points for the cluster-level analysis, using the same methods as described above for the unadjusted analysis. For continuous outcomes, For the two-

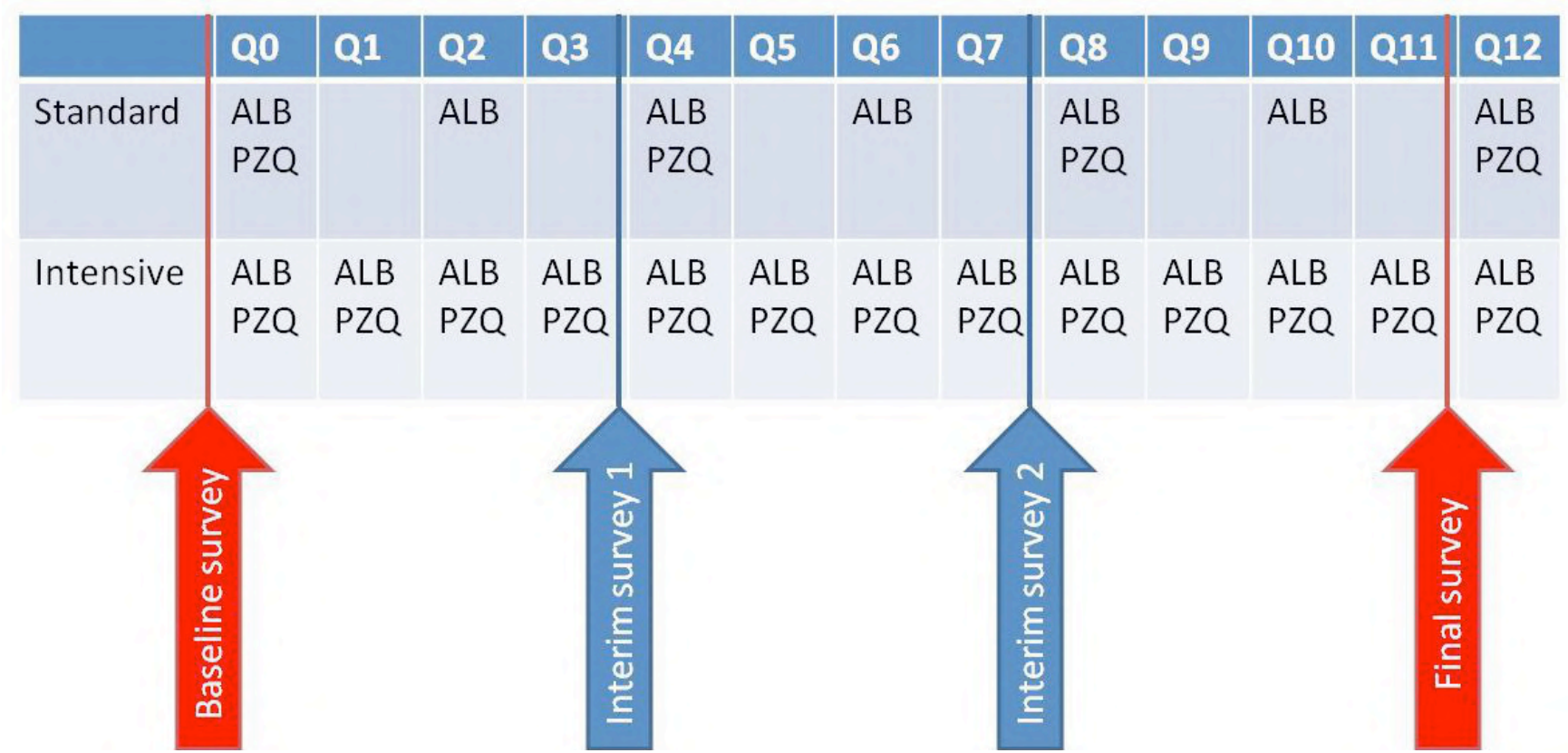
stage adjusted analysis, linear regression was used in the first stage and mean difference-residuals were data points for the second stage.

9.5.2 Supplementary Results

9.5.2.1 Comparison of characteristics of survey participants and non-participants

There were 3566 household members aged one year or above, resident in the 1419 participating households. Of these males were less likely to participate in the questionnaire than females (90% versus 97%), or to give blood (80% versus 87%), stool (75% versus 81%), urine (79% versus 84%) samples or undergo SPT examination (82% versus 89%) (all $p<0.001$). In both arms low numbers of school-aged children were surveyed because of absence, on the mainland, for schooling. Of those surveyed, working age (15-45 years) and pre-school aged (under 5 years) members were less likely to provide samples or undergo SPT ($p<0.001$) than other age groups (working age 81%, 74% and 82% for blood, stool and SPT, respectively; pre-school age 74%, 78% and 87%, respectively; other age groups 89%, 86% and 92%, respectively). Younger age groups were under-represented in the subgroup for whom ImmunoCAP assays were done (162 (12%) of <20 year olds versus 618 (28%) of ≥20 year olds, $p<0.001$), due to lower amounts of available plasma.

Supplementary Figure. Trial treatment and survey timeline



Supplementary Table 1. Impact of intensive versus standard anthelminthic treatment on primary outcomes, restricting to those who had lived in their village throughout (or been born into their village during) the three-year intervention period ("per protocol" analysis)

	n/N (%) / geometric mean		Unadjusted		Adjusted for outcome at baseline, age and sex[5]	
Outcome	Standard	Intensive	RR/GMR (95% CI)	p-value	RR/GMR (95% CI)	p-value
Wheeze (age≥5 years)[1]	29/956 (3.0%)	36/960 (3.8%)	1.23 (0.75, 2.02)	0.40	1.14 (0.66, 1.95)	0.63
Wheeze (age<5 years)	6/234 (2.6%)	3/210 (1.4%)				
Atopy (SPT)						
SPT positivity to any allergen	194/1065 (18.2%)	220/1063 (20.7%)	1.15 (0.84, 1.57)	0.37	1.15 (0.86, 1.55)	0.33
SPT positivity to *Dermatophagoides*	114/1065 (10.7%)	126/1063 (11.9%)	1.10 (0.76, 1.59)	0.59	1.13 (0.80, 1.61)	0.47
SPT positivity to *Blomia tropicalis*	71/1065 (6.7%)	97/1062 (9.1%)	1.41 (0.88, 2.24)	0.14	1.44 (0.91, 2.26)	0.11
SPT positivity to German cockroach	106/1064 (10.0%)	144/1062 (13.6%)	1.41 (0.95, 2.07)	0.07	1.38 (0.94, 2.02)	0.08
Atopy (IgE detected by ImmunoCAP)						
Dermatophagoides or cockroach positivity (>0.35kUa/L)	157/280 (56.1%)	146/259 (56.4%)	1.00 (0.80, 1.24)	0.97	0.99 (0.79, 1.24)	0.95
Dermatophagoides positivity (asIgE>0.35kUa/L)	105/280 (37.5%)	94/259 (36.3%)	0.95 (0.75, 1.20)	0.64	0.95 (0.75, 1.20)	0.63
German cockroach positivity (asIgE>0.35kUa/L)	148/280 (52.9%)	131/259 (50.6%)	0.95 (0.74, 1.21)	0.63	0.95 (0.74, 1.21)	0.66
Concentration of asIgE to *Dermatophagoides* (kUa/L)[2]	GM: 0.170	GM: 0.149	0.84 (0.51, 1.37)	0.46	0.84 (0.52, 1.37)	0.47
Concentration of asIgE to German cockroach (kUa/L)[2]	GM: 0.336	GM: 0.297	0.84 (0.53, 1.34)	0.45	0.86 (0.54, 1.35)	0.49
Atopy (IgE detected by in house ELISA)						
Concentration of asIgE to *Dermatophagoides*[4]	GM: 59.9	GM: 74.1	1.10 (0.33, 3.67)	0.87	1.18 (0.37, 3.76)	0.77
Concentration of asIgE to German cockroach[4]	GM: 56.9	GM: 155	2.05 (0.59, 7.17)	0.25	1.63 (0.47, 5.72)	0.43

[1]For this outcome, a natural log transformation was applied to village level proportions to correct skewed distributions; [2] log10(+0.001) transformation at individual level; [3] log10(+1) transformation at individual level; [4]log10(+1) transformation at individual level; [5]Atopy outcomes assessed by IgE were adjusted for age and sex only; RR: risk ratio; GM: geometric mean; GMR: geometric mean ratio; CI: confidence interval

Supplementary Table 2. Impact of intensive versus standard anthelminthic treatment on primary outcomes, stratified by age group (<4 years, ≥4 years)

	<4 years		≥4 years		
Outcome	RR/GMR (95% CI)	p-value	RR/GMR (95% CI)	p-value	Interaction p-value
Atopy (SPT)	(n=382)		(n=2655)		
SPT positivity to any allergen	1.63 (0.68, 3.91)	0.23	1.07 (0.81, 1.41)	0.63	0.57
SPT positivity to *Dermatophagoides*	1.49 (0.41, 5.34)	0.51	0.97 (0.71, 1.33)	0.84	0.48
SPT positivity to *Blomia tropicalis*	1.44 (0.48, 4.27)	0.50	1.26 (0.82, 1.94)	0.28	0.83
SPT positivity to German cockroach	2.12 (0.37, 12.32)	0.35	1.22 (0.86, 1.73)	0.24	0.79
Atopy (IgE detected by ImmunoCAP)	(n=44)		(n=736)		
Dermatophagoides or cockroach positivity (>0.35kUa/L)	1.38 (0.26, 7.34)	0.72	0.96 (0.84, 1.11)	0.60	0.69
Dermatophagoides positivity (asIgE>0.35kUa/L)	0.55 (0.05, 6.73)	0.61	0.95 (0.76, 1.19)	0.65	0.88
German cockroach positivity (asIgE>0.35kUa/L)	2.41 (0.37, 15.71)	0.41	0.93 (0.80, 1.10)	0.39	0.39
Concentration of asIgE to *Dermatophagoides* (kUa/L)	0.43 (0.10, 1.80)	0.24	0.77 (0.50, 1.17)	0.21	0.43
Concentration of asIgE to German cockroach (kUa/L)	0.95 (0.27, 3.30)	0.93	0.80 (0.55, 1.16)	0.22	0.91
Atopy (IgE detected by in house ELISA)	(n=309)		(n=2646)		
Concentration of asIgE to *Dermatophagoides*	0.94 (0.18, 4.98)	0.94	1.17 (0.38, 3.58)	0.78	0.70
Concentration of asIgE to German cockroach	1.77 (0.36, 8.69)	0.46	2.03 (0.63, 6.53)	0.23	0.69

Supplementary Table 3. Impact of intensive versus standard anthelminthic treatment on schistosomiasis-related liver and spleen morbidity assessed by ultrasonography, in children<18 years

	n (%)		Unadjusted		Adjusted for age and sex	
Outcome	Standard (n=414)	Intensive (n=488)	OR (95% CI)	p-value	OR (95% CI)	p-value
Liver size left lobe[1]						
Normal	171 (41.3%)	212 (43.4%)				
Enlarged	212 (51.2%)	229 (46.9%)				
Much enlarged	31 (7.5%)	47 (9.6%)	0.97 (0.58, 1.61)	0.90	1.05 (0.63, 1.72)	0.86
Spleen size[1]						
Normal	248 (59.9%)	302 (61.9%)				
Moderate splenomegaly	135 (32.6%)	142 (29.1%)				
Marked splenomegaly	31 (7.5%)	44 (9.0%)	0.87 (0.40, 1.89)	0.71	0.86 (0.40, 1.86)	0.69
Portal vein score[2]						
Normal	319 (77.1%)	383 (78.5%)				
Dilation	94 (22.7%)	103 (21.1%)				
Marked dilation	1 (0.2%)	2 (0.4%)	0.70 (0.40, 1.24)	0.23	0.84 (0.50, 1.42)	0.51
Degree of hepatic fibrosis[3]						
Normal	386 (93.7%)	445 (91.4%)				
Peri-portal fibrosis	26 (6.3%)	42 (8.6%)	1.52 (0.75, 3.07)	0.26	1.45 (0.72, 2.94)	0.31

Measurements from ultrasonography were classified according to the Niamey protocol, taking height into account; [1]For liver size left lobe and spleen size, ORs were calculated using a proportional odds model; [2]Portal vein score was analysed as a binary variable, combining dilated and marked dilated categories; [3]Degree of hepatic fibrosis was classified as normal (Niamey protocol image pattern A) or peri-portal fibrosis (Niamey protocol image patterns B-F) and analysed as a binary variable, degree of hepatic fibrosis missing for 2 children in standard arm, 1 child in intensive arm

Supplementary Table 4. Impact of intensive versus standard anthelminthic treatment on helminths, clinical outcomes, hepatosplenomegaly by palpation, and anthropometry, restricting to those who had lived in their village throughout (or been born into their village during) the three-year intervention period ("per protocol" analysis)

	n/N (%) / arithmetic mean		Unadjusted		Adjusted for baseline value, age and sex	
Outcome	Standard	Intensive	RR/mean difference (95% CI)	p-value	RR/mean difference (95% CI)	p-value
Helminth infections						
Schistosoma mansoni, stool Kato Katz	373/965 (38.7%)	225/989 (22.8%)	0.62 (0.41, 0.94)	0.02	0.69 (0.53, 0.90)	0.005
Schistosoma mansoni, stool PCR	576/964 (59.8%)	367/987 (37.2%)	0.65 (0.48, 0.88)	0.006	0.73 (0.62, 0.87)	0.001
Schistosoma mansoni, urine CCA	872/1015 (85.9%)	838/1002 (83.6%)	0.98 (0.90, 1.07)	0.61	0.99 (0.92, 1.07)	0.81
Hookworm, stool PCR[1]	84/964 (8.7%)	62/987 (6.3%)	0.56 (0.27, 1.19)	0.12	0.58 (0.29, 1.13)	0.10
Strongyloides stercoralis, stool PCR	84/964 (8.7%)	51/987 (5.2%)	0.63 (0.40, 0.99)	0.04	0.68 (0.44, 1.04)	0.07
Trichuris trichiura, stool Kato Katz[1]	100/965 (10.4%)	79/989 (8.0%)	0.84 (0.38, 1.84)	0.64	0.77 (0.48, 1.23)	0.26
Ascaris lumbricoides, stool Kato Katz	8/965 (0.8%)	3/989 (0.3%)				
Clinical outcomes						
Visible flexural dermatitis	1/1099 (0.1%)	1/1085 (0.1%)				
Haemoglobin	14.0	13.9	-0.12 (-0.47, 0.22)	0.48	-0.01 (-0.25, 0.24)	0.96
Anthropometry						
Height-for-age z-score, age 1-19 years	-0.39	-0.42	0.05 (-0.21, 0.30)	0.71	0.09 (-0.16, 0.34)	0.45
Weight-for-age z-score, age 1-10 years	-0.09	-0.13	-0.08 (-0.31, 0.16)	0.51	-0.05 (-0.23, 0.12)	0.52
Weight-for-height z-score, age 1-5 years	0.12	0.16	-0.08 (-0.47, 0.30)	0.65	-0.06 (-0.44, 0.31)	0.73
Hepatosplenomegaly, palpation						
Hepatomegaly, palpation	84/1088 (7.7%)	78/1080 (7.2%)	0.93 (0.65, 1.33)	0.69	0.93 (0.64, 1.35)	0.68
Splenomegaly, palpation	59/1090 (5.4%)	40/1081 (3.7%)	0.72 (0.40, 1.31)	0.22	0.72 (0.40, 1.30)	0.22
Hepatosplenomegaly, palpation[1]	14/1089 (1.3%)	8/1082 (0.7%)				
Reported clinical outcomes (exploratory)						
Urticaria, last 12 months	108/1190 (9.1%)	127/1170 (10.9%)	1.20 (0.91, 1.58)	0.19	1.20 (0.94, 1.53)	0.13
Rhinitis, last 12 months	58/1190 (4.9%)	47/1170 (4.0%)	0.89 (0.60, 1.32)	0.56	0.87 (0.58, 1.31)	0.50

[1] For this outcome, a natural log transformation was applied to village level proportions to correct skewed distributions; RR: Risk Ratio; CI: confidence interval; CCA: circulating cathodic antigen; PCR: polymerase chain reaction

Supplementary Table 5: CONSORT 2010 checklist of information to include when reporting a cluster randomised trial

Section/Topic	Item No	Standard Checklist item	Extension for cluster designs	Page No *
Title and abstract				
	1a	Identification as a randomised trial in the title	Identification as a cluster randomised trial in the title	Page 1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	See table 2	Pages 5-6
Introduction				
Background and objectives	2a	Scientific background and explanation of rationale	Rationale for using a cluster design	Pages 7-8
	2b	Specific objectives or hypotheses	Whether objectives pertain to the cluster level, the individual participant level or both	Pages 7-8
Methods				
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Definition of cluster and description of how the design features apply to the clusters	Page 8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons		NA
Participants	4a	Eligibility criteria for participants	Eligibility criteria for clusters	Pages 8-9
	4b	Settings and locations where the data were collected		Page 8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Whether interventions pertain to the cluster level, the individual participant level or both	Page 8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Whether outcome measures pertain to the cluster level, the individual participant level or both	Pages 9-10, Supplementary Methods
	6b	Any changes to trial outcomes after the trial commenced, with reasons		Page 10
Sample size	7a	How sample size was determined	Method of calculation, number of clusters(s) (and whether equal or unequal	Page 10, Supplementary Methods

			cluster sizes are assumed), cluster size, a coefficient of intracluster correlation (ICC or *k*), and an indication of its uncertainty	
	7b	When applicable, explanation of any interim analyses and stopping guidelines		N/A
Randomisation:				
Sequence generation	8a	Method used to generate the random allocation sequence		Page 10, Supplementary Methods
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Details of stratification or matching if used	Page 10, Supplementary Methods
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Specification that allocation was based on clusters rather than individuals and whether allocation concealment (if any) was at the cluster level, the individual participant level or both	Page 10, Supplementary Methods
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Replace by 10a, 10b and 10c	
	10a		Who generated the random allocation sequence, who enrolled clusters, and who assigned clusters to interventions	Page 10, Supplementary Methods
	10b		Mechanism by which individual participants were included in clusters for the purposes of the trial (such as complete enumeration, random sampling)	Pages 9-10
	10c		From whom consent was sought (representatives of the cluster, or individual cluster members, or both), and whether consent was sought before or after randomisation	Page 9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those		N/A

		assessing outcomes) and how		
	11b	If relevant, description of the similarity of interventions		N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	How clustering was taken into account	Pages 10-11, Supplementary Methods
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses		Page 11, Supplementary Methods
Results				
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	For each group, the numbers of clusters that were randomly assigned, received intended treatment, and were analysed for the primary outcome	Pages 11-12, Figure 2
	13b	For each group, losses and exclusions after randomisation, together with reasons	For each group, losses and exclusions for both clusters and individual cluster members	N/A
Recruitment	14a	Dates defining the periods of recruitment and follow-up		Pages 11-12
	14b	Why the trial ended or was stopped		N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Baseline characteristics for the individual and cluster levels as applicable for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	For each group, number of clusters included in each analysis	Figure 2, Tables 2 and 3, Page 12
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Results at the individual or cluster level as applicable and a coefficient of intracluster correlation (ICC or k) for each primary outcome	Pages 12-13, Tables 2 and 3, Supplementary Table 3
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended		
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses,		Pages 12-13, Supplementary Tables 1, 2 and 4

		distinguishing pre-specified from exploratory		
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)		Pages 13-14
Discussion				
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses		Pages 14-16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Generalisability to clusters and/or individual participants (as relevant)	Pages 15-16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence		Pages 14-16
Other information				
Registration	23	Registration number and name of trial registry		Page 5
Protocol	24	Where the full trial protocol can be accessed, if available		Page 8
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders		Page 17

** Note: page numbers optional depending on journal requirements*

1. Ellwood P, Asher MI, Beasley R, Clayton TO, Stewart AW. ISAAC Phase Three Manual. ISAAC International Data Centre, Auckland, New Zealand, 2000.

2. Williams HC. So how do I define Atopic Eczema? A practical manual for researchers wishing to define atopic eczema. http://www.nottingham.ac.uk/dermatology/eczema/index.html (accessed 15th September 2010.

3. WHO. Ultrasound in schistosomiasis: a practical guide to the standardized use of ultrasonography for the assessment of schistosomiasis-related morbidity2000. http://apps.who.int/iris/bitstream/10665/66535/1/TDR_STR_SCH_00.1.pdf (accessed 28th May 2017).

4. Mpairwe H, Webb EL, Muhangi L, et al. Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. *Pediatr Allergy Immunol* 2011; **22**(3): 305-12.

5. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Revista do Instituto de Medicina Tropical de Sao Paulo* 1972; **14**(6): 397-400.

6. Verweij JJ, Canales M, Polman K, et al. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2009; **103**(4): 342-6.

7. Emmanuel IO, Ekkehard D. Epidemiology, of bilharzias (schistosomiasis) in Uganda from 1902 until 2005. *African health sciences* 2008; **8**(4): 239-43.

8. Melrose WD, Turner PF, Pisters P, Turner B. An improved Knott's concentration test for the detection of microfilariae. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2000; **94**(2): 176.

9. Nampijja M, Webb EL, Kaweesa J, et al. The Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA): study protocol for a randomised controlled trial. *Trials* 2015; **16**: 187.

9.6 APPENDIX 4: Copies of ethical approvals

9.6.1 Copy of ethical approval to conduct PhD studies at LSHTM

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

Observational / Interventions Research Ethics Committee

Mr NKURUNUNGI GYAVIIRA

LSHTM

23 November 2016

Dear NKURUNUNGI

Study Title: Immunological mechanisms of helminth-allergy associations in Uganda

LSHTM Ethics Ref: 11838

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation. The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Advertisements	1. LaVIISWA. Information and permission, LC committees_original	08/03/2012	1
Local Approval	UVRI REC LaVIISWA Approval april 2012	02/05/2012	01
Local Approval	UNCST LaVIISWA Approvals 2012-2015	25/05/2012	01

Local Approval	LSHTM LaVIISWA Approvals 2012-2016	28/05/2012	01
Information Sheet	Info and consent form, adults, LaVIISWA main Surveys v 3.2	05/03/2013	3.2
Information Sheet	Info and consent form,adults, LaVIISWA main Surveys v 3.2 Luganda	05/03/2013	3.2
Information Sheet	Info and consent form, parents and guardians, LaVIISWA main surveys v 3.2	05/03/2013	3.2
Information Sheet	Info and consent form, parents and guardians, LaVIISWA main survey v 3.2, Luganda	05/03/2013	3.2
Information Sheet	Info and Assent form, LaVIISWA main Survey, v 3.2	05/03/2013	3.2
Information Sheet	Info and Assent form, LaVIISWA main survey, v 3.2, Luganda	05/03/2013	3.2
Local Approval	UNCST LaVIISWA APPROVAL_Immunological mechanisms_sept 2015	25/09/2015	01
Local Approval	LAVIISWA MTA approval UNCST	15/10/2015	01
Information Sheet	LAVIISWA Inf for adult members - English	05/01/2016	5
Information Sheet	LAVIISWA Inf for adult members - Luganda	05/01/2016	5
Information Sheet	LAVIISWA Inf for children - English	05/01/2016	5
Information Sheet	LAVIISWA Inf for children - Luganda	05/01/2016	5
Information Sheet	LAVIISWA Inf for parents & guardians - English	05/01/2016	5
Information Sheet	LAVIISWA Inf for parents & guardians - Luganda	05/01/2016	5
Information Sheet	Urban Survey Information and Assent for children _Storage of Blood Samples English	08/03/2016	3
Information Sheet	Urban Survey Information and Assent for children _Storage of Blood Samples Luganda	08/03/2016	3
Information Sheet	Urban Survey Information and Assent for children English	08/03/2016	3
Information Sheet	Urban Survey Information and Assent for children Luganda	08/03/2016	3
Information Sheet	Urban Survey Information and Consent for adults _Storage of Blood Samples English	08/03/2016	3
Information Sheet	Urban Survey Information and Consent for adults _Storage of Blood Samples Luganda	08/03/2016	3
Information Sheet	Urban Survey Information and Consent for adults English	08/03/2016	3
Information Sheet	Urban Survey Information and Consent for adults Luganda	08/03/2016	3
Information Sheet	Urban Survey Information and Consent for Parents & Guardians _Storage of Blood Samples English	08/03/2016	3
Information Sheet	Urban Survey Information and Consent for Parents & Guardians _Storage of Blood Samples Luganda	08/03/2016	3
Information Sheet	Urban Survey Information and Consent for Parents & Guardians English	08/03/2016	3
Information Sheet	Urban Survey Information and Consent for Parents & Guardians Luganda	08/03/2016	3
Local Approval	LAVIISWA UNCST Approval of transfer of samples to Cambridge University UK[2]	26/04/2016	01
Local Approval	Urban survey_approvals	26/07/2016	01
Local Approval	LSHTM Letter _Urban Survey	26/07/2016	01

Protocol / Proposal	Gyaviira Nkurunungi_protocol_2016	29/07/2016	01
Protocol / Proposal	Gyaviira Nkurunungi_data management plan	29/07/2016	1
Investigator CV	Curriculum vitae_Gyaviira Nkurunungi_08_2016	23/08/2016	01
Covering Letter	Cover letter_Gyaviira Nkurunungi_ref11838_final	08/11/2016	01

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

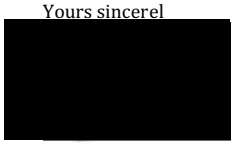
At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the

committee via the website at: http://leo.lshtm.ac.uk Additional information is available at:

www.lshtm.ac.uk/ethics

Yours sincerel



Professor John DH Porter
Chair
ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

9.6.2 *The Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA): copies of ethical approvals**

Tel: (256) 41- 321962 (Direct)
(256) 41- 320385/6 (General)
Fax: (256) 41- 320483
Email:directoruvri@uvri.go.ug

THE REPUBLIC OF UGANDA

UGANDA VIRUS RESEARCH INSTITUTE
P.O.Box 49 ENTEBBE (U)

Our Ref : GC/127/12/05/03
Your Ref:

2nd May 2012

Prof Alison Elliot,

RE: UVRI SEC review of protocol titled; "**Lake Victoria Island Intervention Study on Worms and Allergy-related disease (LaVIISWA).**"

Thank you for your responses to the queries addressed to you by UVRI Science and Ethics Committee (SEC) during its April 19th 2012 meeting.

This is to inform you that your response dated 24th April 2012 was reviewed and met the requirements of the UVRI Science and Ethics Committee (SEC).

UVRI SEC annual approval has been given for you to conduct your research up to 2nd May 2013. Annual progress report and request for extension should be submitted to UVRI SEC prior to the expiry date, to allow timely review.

The reviewed and approved documents included;

- Study Protocol.
- Material Transfer agreement
- Draft Questionnaires
- Information and consent documents
- CVs of External collaborators.

You can now commence with your study after registration with the Uganda National Council for Science and Technology (UNCST).

Note: UVRI SEC requires you to submit a copy of the UNCST approval letter for the above study before commencement.

Yours sincerely,

Tom Lutalo
Chair, UVRI SEC

C.C Secretary, UVRI SEC

*Approvals for continuation of the study sought for, and obtained each year.

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LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Alison Elliott
Professor of Tropical Medicine
CRD/ITD
LSHTM

28 May 2012

Dear Professor Elliott

Study Title: **Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA)**
LSHTM ethics ref: **6187**

Thank you for your application of 14 April 2012 for the above research, which has now been considered by the Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
LSHTM ethics application	n/a	
Protocol	V1	08/03/2012
Information Sheet	V1	08/03/2012
Consent form	V1	08/03/2012

After ethical review

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form.

Yours sincerely,

[redacted]

Professor Andrew J Hall
Chair

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 1183 **21 August 2012**

Dr. Allison Elliott
MRC/UVRI Uganda Research Unit on AIDS
Uganda Virus Research Institute
P.O Box 49
ENTEBBE

Dear Dr. Elliott,

RE: RESEARCH PROJECT, "LAKE VICTORIA ISLAND INTERVENTION STUDY ON WORMS AND ALLEGY-RELATED DISEASES (LaVIISWA)"

This is to inform you that on 20th August 2012, Uganda National Council for Science and Technology (UNCST) reviewed and approved the revision made to the above study. The approved changes include:

1. To add lung function testing by simple spirometry at rest to complement the other study procedures.
2. Correction of a typographical error about the amount of blood to be collected from 4 year olds and those between 5-12 years.

The approval covers the following attachments:
a. Revised LaVIISWA proposal version 2 dated 3 July 2012.
b. Revised consent and assent forms for the main surveys version dated 3 July 2012.

The study is valid until **23 May 2013**. If however, it is necessary to continue with the study beyond the expiry date, a request for continuation should be made to the Executive Secretary, UNCST

Yours sincerely,

[redacted]

Leah Nawegulo
for: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500, (256) 312 314800
FAX: (256) 414-234579
EMAIL: infc@uncst.go.ug
WEBSITE: http://www.uncst.go.ug

9.6.3 *The Urban survey of Allergy-related and Metabolic Outcomes: copies of ethical approvals**

UNHRO
Uganda National Health Research Organisation

Uganda Virus Research Institute
Plot 51-59, Nakiwogo Road, Entebbe
P.O. Box 49, Entebbe-Uganda
Tel: +256 414 320 385 / 6
Fax: +256 414 320 483
Email: directoruvri@uvri.go.ug

REPUBLIC OF UGANDA

Our Ref: GC/127/16/02/547
Your Ref:

23rd February 2016

Prof. Alison Elliott,

RE: UVRI REC review of protocol titled **"The Entebbe Urban Survey on Allergy-related and Metabolic Outcomes."**

Thank you for submitting the above Protocol dated 10th January 2016 to the UVRI Research Ethics Committee (REC).

This is to inform you that your Protocol was reviewed and members had the following queries for you to address;

a) The Novak et al urbanicity scale variables do not progress in the same way in all environments. Koome sewage system and road network may not be different from that of Kigungu and Katabi wards. The lower level [H/C 11 and 111] health system in Entebbe Municipality could be almost same as that of Koome Island. There is need for more detail on how the urbanicity scale will be utilized and which score will they use to define urbanicity other than quoting Novak et al.
b) REC feels that the time spent answering the study questionnaires should be compensated.
c) Members suggested that Malaria should also be treated.
d) Please provide a separate consent for storage of samples as this is a requirement by the UNCST.

Chairman's Action will suffice once the above concerns are addressed.

Yours sincerely

[redacted]

Mr. Tom Lutalo
Chair, UVRI REC
C.C Secretary, UVRI REC

*Approvals for continuation of the study sought for, and obtained each year.

UNHRO
Uganda National Health Research Organisation

Uganda Virus Research Institute
Plot 51-59, Nakiwogo Road, Entebbe
P.O. Box 49, Entebbe-Uganda
Tel: +256 414 320 385 / 6
Fax: +256 414 320 483
Email: directoruvri@uvri.go.ug

REPUBLIC OF UGANDA

Our Ref: GC/127/17/04/547

Your Ref:

April 4, 2017

Dear Prof. Alison Elliott,

RE: UVRI REC review of progress report titled "**Entebbe Urban Survey on Allergy-related and Metabolic Outcomes**"

Thank you for submitting your progress report for the above study dated March 29, 2017 to the UVRI Research Ethics Committee (REC).

This is to inform you that after review of your report, UVRI REC continuation approval has been granted for you to continue with this study up to March 31, 2018.

At that time, REC would expect you to submit a progress report and request for renewal, prior to the expiry date, to allow timely review.

Yours sincerely,

[redacted]

Mr. Tom Lutalo
Chair, UVRI REC
C.C Secretary, UVRI REC

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United Kingdom
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LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Professor Alison Elliott
Professor of Tropical Medicine
Department of Clinical Research (CRD)
LSHTM

7 June 2016

Dear Professor Alison

Study Title: THE ENTEBBE URBAN SURVEY OF ALLERGY-RELATED AND METABOLIC OUTCOMES (USAMO)

LSHTM Ethics Ref: '10709- 1'

Thank you for your application for the above amendment to the existing ethically approved study and submitting revised documentation. The amendment application has been considered by the Observational Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above amendment to research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval for the amendment having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Other	3. Urban survey protocol_8March_2016_Track changes_ae	08/03/2016	0.3
Other	3. Urban survey protocol_8March_2016_clean copy_ae	08/03/2016	0.3
Other	4. Info and Assent form, URBAN SURVEY, English_storage added	08/03/2016	0.3
Other	5. Info and Consent form, adults, URBAN SURVEY, English V2_storage added	08/03/2016	0.3
Other	6. Info and Consent form, parents and guardians, URBAN SURVEY, English_storage added	08/03/2016	0.3
Other	7. Info and Consent form, adults, URBAN SURVEY, Luganda_storage added	08/03/2016	0.3
Other	8. Info and consent form, parents-guardians, URBAN SURVEY, Luganda_storage added	08/03/2016	0.3
Other	9. Info and Assent form, URBAN SURVEY-Luganda_storage added	08/03/2016	0.3
Other	Entebbe Urban Survey initial UVRI REC Approval	31/03/2016	n/a

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

[redacted]

Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 2036 **7th June 2016**

Alison Elliot
Principal Investigator
MRC/UVRI Uganda
Research Unit on AIDS
Entebbe

Re: Research Approval: **The Entebbe Urban Survey of Allergy-Related and Metabolic Outcomes (USAMO)**

I am pleased to inform you that on **17/05/2016**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period **17/05/2016** to **17/05/2017.**

Your research registration number with the UNCST is **HS 2036.** Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local REC for review with copies to the National Drug Authority.
4. Unexpected events involving risks to research subjects/participants must be reported promptly to the UNCST. New information that becomes available which alters the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. A progress report must be submitted electronically to UNCST within four weeks after every 12 months. Failure to do so may result in termination of the research project.

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
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COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: http://www.uncst.go.ug

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	0.3	March 2016
2.	Consent for Storage of Blood Samples	English and Luganda	0.3	March 2016
3.	Information for Children	English and Luganda	0.3	March 2016
4.	Information for Parents/Guardians of Child Household Members	English and Luganda	0.3	March 2016
5.	Information for Adult Household Members	English and Luganda	0.3	March 2016
6.	Urban Survey Village Questionnaire	English and Luganda	1.0	April 2016
7.	Urban Survey Individual Questionnaire (Children and Adults Aged 13 years or Above)	English and Luganda	2.0	April 2016
8.	Urban Survey Individual Questionnaire (Children Aged 12 Years or below)	English	2.0	April 2016
9.	Urban Survey Individual Questionnaire (Children Aged 12 Years or below)	Luganda	1.0	January 2016
10.	Urban Survey Household Questionnaire	English and Luganda	2.0	April 2016

Yours sincerely,

[redacted]

Hellen. N. Opolot
for: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

cc. Chair, Uganda Virus Research Institute, Research Ethics Committee

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

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9.6.4 Study on Asthma and Parasitic Infections: copies of ethical approvals

UNHRO
Uganda National Health Research Organisation

Uganda Virus Research Institute

Plot 51-59, Nakiwogo Road, Entebbe
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Tel: +256 414 320 385 / 6
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REPUBLIC OF UGANDA

Our Ref: GC/127/14/09/481
Your Ref: .....

09th September 2014

Dr. Harriet Mpairwe,

RE: UVRI REC review of protocol titled **"Study on asthma and parasitic infections among children in Uganda (SONA)."**

Thank you for submitting your responses to the queries addressed to you by UVRI REC.

This is to inform you that your responses dated 04th September 2014 were reviewed and met the requirements of the UVRI Research Ethics Committee.

UVRI REC annual approval has been given for you to conduct your research up to 09th September 2015. Annual progress report and request for extension should be submitted to UVRI REC prior to the expiry date, to allow timely review.

The reviewed and approved documents included;

1. Study protocol Version 1_4 Sept 2014
2. UVRI REC Application form
3. Consent forms and information sheets
4. Screening questionnaires
5. Applicants' CVs.

You can now continue with your study after registration with the Uganda National Council for Science and Technology (UNCST).

Note: UVRI REC requires you to submit a copy of the UNCST approval letter for the above study before commencement.

Yours sincerely,

[redacted]

Mr. Tom Lutalo
Chair, UVRI REC
C.C The Director-UVRI
C.C Secretary, UVRI REC

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REPUBLIC OF UGANDA

Our Ref: GC/127/16/08/350
Your Ref:

August 18, 2016

Dr. Harriet Mpairwe,

RE: UVRI REC review of progress report titled "**Study on asthma and parasitic infections among children in Uganda (SONA)".**

Thank you for submitting your progress report for the above study dated August 18, 2016 to the UVRI Research Ethics Committee (REC).

This is to inform you that after review of your report, UVRI REC continuation approval has been granted for you to continue with this study up to September 1, 2017.

At that time, REC would expect you to submit a progress report and request for renewal, prior to the expiry date, to allow timely review.

Yours sincerely,

[redacted]

Mr. Tom Lutalo
Chair, UVRI REC
C.C Secretary, UVRI REC

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 1707

20/11/2014

Dr. Harriet Mpairwe
MRC/UVRI Uganda Research Unit on AIDS
Entebbe

Re: Research Approval: **Study on Asthma and Parasitic Infections among Children in Uganda**

I am pleased to inform you that on **11/11/2014**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **11/11/2014** to **11/11/2017**.

Your research registration number with the UNCST is **HS 1707**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated local Institutional Review Committee (IRC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local IRC for review with copies to the National Drug Authority.
4. Unanticipated problems involving risks to research subjects/participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. A progress report must be submitted electronically to UNCST within four weeks after every 12 months. Failure to do so may result in termination of the research project.

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1	Research Proposal	English	1	4/09/2014
2	Screening Questionnaires	English, Luganda	1	20/07/2014
3	Informed Consent Form	English, Luganda	1	4/09/2014
4	Informed Assent Form	English, Luganda	1	4/09/2014
5	Information Sheet and Consent Form	English, Luganda	1	4/09/2014
6	Information Sheet and Assent Form	English, Luganda	1	4/09/2014
7	Consent for Storage	English, Luganda	1	4/09/2014

Yours sincerely,

[redacted]

Winfred Badanga
for: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY
cc Chair, Uganda Virus Research Institute Research Ethics Committee

LOCATION/CORRESPONDENCE

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9.7 APPENDIX 5: example of a Stata do file used to perform permutation testing

```
*************************PERMUTATION TESTING**********************

**This is to assess differences between comparison groups, by controlling
**for multiple testing of these correlated (non-independent) anti-glycan
**responses. It is an alternative to other methods of accounting for multiple
**testing that assume that variables are independent of each other, such as
**Bonferroni corrections.

** The code for analysing the data and outputting p-values to a matrix, and
**then to a dataset, uses a Mann whitney U-test here (note that this can
**easily be adapted to other tests, such as regression if adjusting for
**confounders). Here we assess differences between S. mansoni infected and
**uninfected individuals. Variable name for S. mansoni infection is 'kasinf'.
**The outcomes are IgE responses to the 135 glycans on the array (from G1 to
**G136).

** loop through the outcome variables from G1 to G136. When you see `x' it
takes values from 1 to 136.

foreach x of varlist G1-G136 {

** conduct a Mann whitney test

ranksum `x', by(kasinf)

** then extract the p-value

scalar p = 2 * normprob(-abs(r(z)))
di p

** add this p-value to a matrix, so have all the p-values from a single
** analysis of all outcomes, in one place.

if `x'==G1 matrix pval_vec=p
else matrix pval_vec=(pval_vec \ p)
}

*** Look at the output

matrix list pval_vec

**This gives the p-values from tests of association between exposure and
**outcome for all 135 outcomes. These are our actual results.
** We want to do this thousands of times, but rather than using the original
** dataset, we want to "muddle up" the exposure variable so that we destroy
** any genuine associations between exposure and the outcome.
** The idea is that we can then see, under the null hypothesis of no
**association, how many times we would expect to see a result with this small
**a p-value by chance alone.
** For each outcome variable, we can then compare our p-value with the
**calculated p-values from each permutation, to see where our p-value lies in
**relation to these.

** We can also look globally, i.e. from each permutation, we take the
```

```
**smallest p-value and we then compare our smallest p-value (from the true)
**data with the distribution of smallest p-values from all of the
**permutations.

*** Firstly, permute the exposure values and repeat:
** To do this, use a command called shufflevar which needs to be installed

ssc install shufflevar

** Look through 1000 iterations and for each iteration shuffle up the
exposure variable (keeping other data the same).

foreach num of numlist 1(1)1000 {
gen eper`num'=kasinf
shufflevar eper`num', dropold
rename eper`num'_shuffled eper`num'
}

**Now that have the mixed-up exposure labels, need to loop through and apply
the statistical tests to the permuted data.

set more off
foreach y of numlist 1(1)1000 {
foreach x of varlist G1-G136 {

** conduct statistical analysis

ranksum `x', by(eper`y')

** then extract the p-value

scalar p = 2 * normprob(-abs(r(z)))
di p

** add this p-value to a matrix, so have all the p-values from a single
** analysis of all outcomes, in one place.

if `x'==G1 matrix pval_vec`y'=p
else matrix pval_vec`y'=(pval_vec`y' \ p)
}
}

*** Look at the output

matrix dir

**We now have a matrix (with 1 column each) of results for the permuted
**datasets.
** Each has 136 p-values, one corresponding to each outcome variable.
*** Now need to combine the separate matrices so have all in one place.
*** Easiest way to do this is to export as .csv file, then bring back into
**Stata.
** Output true results p-values first.

matrix pval_vect=pval_vec'
mat2txt, matrix(pval_vect) saving("permute_laviiswa_glycan_IgE.csv") replace

** Then output the permutation results.
```

```
foreach x of numlist 1/1000 {
matrix pval_vec`x't=pval_vec`x''
mat2txt, matrix(pval_vec`x't) saving("permute_laviiswa_glycan_IgE.csv")
append
}


** Bring the p-values back into Stata and do some tidying up

import delimited permute_laviiswa_glycan_IgE.csv, clear
drop if v2=="r1"
drop v1
set more off
foreach x of varlist v2-v136 {
destring `x', replace
}

drop v137

** In this dataset, each column represents one of the 30 outcomes.
** The first row represents the true results (from the actual data) - p-
values. Each row after that represents the p-values from one permutation.

** We can generate a new variable for the minimum p-value from each
permutation

egen minp=rowmin(v2-v136)

** We now have the option of comparing our best p-value from the true
analysis with our best p-values from all permutations, or we can compare
results for each outcome variable to their permuted equivalents individually
(or both).

** Globally:

gen global=0 if _n>=2
replace global=1 if minp[1]>minp[_n] & _n>=2

** get p-value from the proportion of permutations that have a p-value
** smaller than the actual p-value

tab global

** We are looking for a small number of 1s, e.g. if there are only 3 1's out
** of 1000 permutations, then the p-value would be 3/1000 = 0.003. If there
were 500 1's out of 1000 permutations, then the p-value would be 0.5.

** For each outcome:

foreach x of numlist 2/136 {
local y=`x'-1
gen kasinf`y'p=0 if _n>=2
replace kasinf`y'p=1 if v`x'[1]>v`x'[_n] & _n>=2
}

set more off
foreach x of varlist kasinf1p-kasinf135p {
tab `x'
}
```